



Litton

# BIONETICS

**N29**

SCREENING STUDIES  
 HOST-MEDIATED ASSAY  
 CYTOGENETICS  
 DOMINANT LETHAL ASSAY  
 CONTRACT FDA 71-268  
 COMPOUND FDA 71-43  
 TALC  
Mg Silicate

Summary of mutagenicity screening studies, host-mediated assay cytogenetics dominant  
 lethal assay-Contract FDA 71-268 & Compound FDA 71-43  
 12/9/74  
 (Talc, Mg Silicate)

LBI PROJECT #2446

~~N29~~  
N29

SUMMARY OF MUTAGENICITY  
SCREENING STUDIES  
HOST-MEDIATED ASSAY  
CYTOGENETICS  
DOMINANT LETHAL ASSAY  
CONTRACT FDA 71-268  
COMPOUND FDA 71-43  
TALC

Mg Silicate

SUBMITTED TO

FOOD & DRUG ADMINISTRATION  
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.  
5516 NICHOLSON LANE  
KENSINGTON, MARYLAND

DECEMBER 9, 1974



**BIONETICS**



**BIONETICS**

5518 Nicholson Lane, Kensington, Maryland 20795 301 881-5800

December 9, 1974

Mr. Leonard Appleby, Contracting Officer  
Department of Health, Education and Welfare  
Public Health Service  
Food and Drug Administration, CA-212  
5600 Fishers Lane, Room 5C13  
Rockville, Maryland 20852

Reference: Contract FDA 71-268; LBI Project #2446

Dear Mr. Appleby:

Litton Bionetics, Inc., is pleased to submit a report for the referenced contract entitled "Mutagenicity Screening Studies" for compound FDA 71-43, Talc.

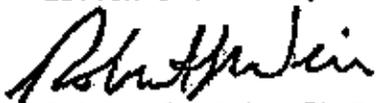
Included in this report are the results and raw data of the three tests conducted: Host-Mediated Assay, Cytogenetic Studies and Dominant Lethal Assay. Eight (8) copies are being submitted for your review.

Upon completion of the toxicology work an evaluation was made of our results to those appearing in the literature. In cases where our values were lower, the toxicology was repeated. In some instances either the Host-Mediated Assay, Dominant Lethal Assay, and/or Cytogenetic Studies were also repeated at one or more levels to fulfill the requirements of the contract. In some cases, the acute and/or subacute assays were involved.

If there are any questions concerning this report, or, if additional information is required, please do not hesitate to contact us.

Sincerely,

LITTON BIONETICS, INC.

  
Robert J. Weir, Ph.D.  
Vice President

RJW:11s  
Enclosures (8)

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## I. REPORT

### A. Introduction

Litton Bionetics, Inc. (LBI) has investigated the possible mutagenicity of compounds selected and provided by the Food and Drug Administration under Contract 71-268. LBI's investigation utilized the three mammalian test systems herein described -- Host-Mediated Assay, Cytogenetic Studies and Dominant Lethal Assay. These tests provide information as to the types of genetic damage caused by environmental compounds -- pesticides, chemicals, food additives, drugs and cosmetics.

The Host-Mediated Assay is based upon the assumption that the action of a mutagen on the genetics of bacteria is similar to that in man. This is further strengthened by the use of an eukaryotic organism (Saccharomyces cerevisiae). Since the mutation frequencies are well established for the indicator organism, any deviation due to the action of the test compound is readily detectable. As some compounds are mutagenic in bacteria and not in the host animal, and vice versa, this test is able to differentiate an action which may have been due to hosts' ability to detoxify or potentiate a suspected mutagen. This action is dependent upon the ability of the compound to gain access to the peritoneal cavity. Coupled with the direct action of the compound on the indicator organism in vitro, the assay provides a clear insight into host-mediation of mutagenicity.

Cytogenetics provides a valuable tool for the direct observation of chromosomal damage in somatic cells. Alteration of the chromosome number and/or form in somatic cells may be an index of mutation. These studies utilized examination of bone marrow cells arrested in C-metaphase from rats exposed to the test compound as compared to positive and negative control animals. If mutational

changes occur, the types of damage expected due to the action of chemicals are structural rearrangements, breaks and other forms of damage to the chromosomal complement of the cells exposed.

For the in vitro cytogenetic studies, we have a more rapid and inexpensive means of determining chromosomal damage. This is accomplished by observing cells in anaphase. As the chromatids separate and move along the spindle, aberrations may occur. Chromatids which do not migrate to the daughter cells may lead to uneven distribution of parts or of entire chromatids (mitotic nondysjunction). These give rise to "side arm" bridges which have been interpreted as point stickiness or localized failures of chromosome duplication point errors. These aberrations (bridges, pseudochiasmata, multipolar cells, acentric fragments, etc.) are extremely sensitive indicators of genetic damage.

The Dominant Lethal Test is an accurate and sensitive measure of the amount and type of fetal wastage which may occur following administration of a potential mutagen. Dominant lethal mutations are indicators of lethal genetic lesions. The effects of mutagens on the chromosomal complement of the spermatozoa of treated males results in alterations of form and number of chromosomes. Structural rearrangements and aneuploidy may lead to the production of non-viable zygotes, early and late fetal deaths, abortions and congenital malformations. In addition, aberrations could lead to sterility or reduced reproductive capacity of the  $F_1$  generation. The action of a mutagen on specific portions of spermatogenesis is also apparent in this test.

#### B. Objective

The purpose of these studies is to determine any mutagenic effect of the test compound by employing the Host-Mediated Assay, Cytogenetic Studies



and the Dominant Lethal Assay, both in vivo and in vitro tests are employed with the cytogenetic and microbial test systems. These tests and their descriptions are referenced in the Appendices A through F.

C. Compound

1. Test Material

Compound FDA 71-43, Talc, Lot Number 11-16-17 (#141), as supplied by the Food and Drug Administration.

2. Dosages

The animals employed, the determination of the dosage levels and the route of administration are contained in the technical discussion.

The dosage levels employed for compound FDA 71-43 are as follows for the Cytogenetic Studies in vivo in rats.

	<u>Test I<sup>+</sup></u>	<u>Test II<sup>+</sup></u>
Low Level	30 mg/kg	-----
Intermediate Level	300 mg/kg	-----
LD <sub>5</sub>	3000 mg/kg	5000 mg/kg
Negative Control	Saline	Saline
Positive Control (TEM*)	0.3 mg/kg	0.3 mg/kg

The dosage levels employed for compound FDA 71-43 are as follows for the Host-Mediated Assay in vivo in mice.

	<u>Test I<sup>+</sup></u>	<u>Test II<sup>+</sup></u>
Low Level	30 mg/kg	-----
Intermediate Level	300 mg/kg	-----
LD <sub>5</sub>	3000 mg/kg	5000 mg/kg
Negative Control	Saline	Saline
Positive Control (EMS**)	350 mg/kg	350 mg/kg
(DMN***)	100 mg/kg	100 mg/kg

\* Triethylene Melamine

\*\* Ethyl Methane Sulfonate

\*\*\* Dimethyl Nitrosamine

+ These two tests were performed at different time intervals.



The dosage levels employed for compound FDA 71-43 are as follows for the Dominant Lethal Assay in vivo in rats.

	<u>Test I</u> <sup>+</sup>	<u>Test II</u> <sup>+</sup>
Low Level	30 mg/kg	-----
Intermediate Level	300 mg/kg	-----
LD <sub>5</sub>	3000 mg/kg	5000 mg/kg
Negative Control	Saline	Saline
Positive Control (TEM*)	0.3 mg/kg	0.3 mg/kg

The in vitro Cytogenetic Studies were performed employing three logarithmic dose levels.

Low Level	2 mcg/ml
Medium Level	20 mcg/ml
High Level	200 mcg/ml
Negative Control	Saline
Positive Control (TEM*)	0.1 mcg/ml

The discussion of this test is contained in the technical discussion.

D. Methods

The protocols employed are explained in Appendices C and D.

E. Summary

1. Host-Mediated Assay

This compound caused no significant increases in mutant or recombinant frequencies with Salmonella TA-1530 and G-46 or Saccharomyces D3, respectively, at the dose levels tested. The in vitro tests were negative.

2. Cytogenetics

a. In vivo

The compound produced no detectable significant aberration of the bone marrow metaphase chromosomes of rats when administered orally at the dosage levels employed in this study.

\*Triethylene Melamine

+These two tests were performed at different time intervals.



b. In vitro

The compound produced no significant aberration in the anaphase chromosomes of human tissue culture cells when tested at the dosage levels employed in this study.

3. Dominant Lethal

This compound was considered to be non-mutagenic in this assay system when used at the dosage levels employed in this study in rats.

F. Results and Discussion

1. Toxicity Data - Test I

a. In vivo

Compound FDA 71-43 was suspended in 0.85% saline and administered to ten male rats by intubation. The average weight of the animals was 250 grams and each received a dose of 5000 mg/kg. All animals were found dead within 24 hours.

Dose levels of 50, 100, 500, 1000 and 2000 mg/kg were selected to determine an acute LD<sub>50</sub>. The toxicity data is presented on the LD<sub>50</sub> reporting form using the Litchfield-Wilcoxon method.

The LD<sub>50</sub> was determined as 920 mg/kg. The LD<sub>5</sub> was derived from the probit line. The dose levels used were: LD<sub>5</sub> - 300 mg/kg, intermediate - 30 mg/kg, and low - 3.0 mg/kg. The data on the dose levels, numbers of animals and necropsy findings are presented in the toxicity data sheets.

b. In vitro

The compound was suspended in 0.85% saline at the concentrations listed above. It was introduced into tubes containing WI-38 cells in a logarithmic phase of growth. The cells were observed for cytopathic



effect (CPE) and the presence of mitosis at 24 and 48 hours.

<u>Tube No.</u>	<u>No. of Cells</u>	<u>Conc. mcg/ml</u>	<u>CPE</u>	<u>Mitosis</u>
1	$5 \times 10^5$	5000	+	-
2	$5 \times 10^5$	5000	+	-
3	$5 \times 10^5$	1000	+	-
4	$5 \times 10^5$	1000	+	-
5	$5 \times 10^5$	500	+	-
6	$5 \times 10^5$	500	+	-
7	$5 \times 10^5$	100	-	+
8	$5 \times 10^5$	100	-	+
9	$5 \times 10^5$	10	-	+
10	$5 \times 10^5$	10	-	+

Since an inhibition of mitosis was observed, a closer range of concentrations were employed as follows.

1	$5 \times 10^5$	500	+	-
2	$5 \times 10^5$	500	+	-
3	$5 \times 10^5$	400	+	-
4	$5 \times 10^5$	400	+	-
5	$5 \times 10^5$	300	-	-
6	$5 \times 10^5$	300	-	+
7	$5 \times 10^5$	200	-	+
8	$5 \times 10^5$	200	-	+
9	$5 \times 10^5$	100	-	+
10	$5 \times 10^5$	100	-	+

The 200 mcg/ml concentration was used as the high level, 20 mcg/ml as the intermediate, and 2.0 mcg/ml as the low.

c. TOXICITY DATA SHEETS  
CONTRACT FDA 71-268  
COMPOUND FDA 71-43  
TALC  
TEST I



TOXICITY DATA  
COMPOUND FDA 71-43

Solvent: 0.85% saline

Dosage Form: Suspension

Animals: Male rats with an average body weight of 250 grams. All animals were observed for ten (10) days.

Range Finding:

	<u>Dose</u> <u>mg/kg</u>	<u># Dead</u> <u># Animals</u>	<u>Day of Death and Necropsy</u>
	5000	10/10	Day 1: Impacted stomach, liver patchy, some bloody fluid in stomach and intestine.
LD <sub>50</sub> :	50	0/5	None
	100	0/5	None
	500	1/5	Day 4: Impacted stomach, liver patchy, some bloody fluid in stomach and intestine.
	1000	3/5	Day 2 (1) and Day 3 (2): Impacted stomach, liver patchy, some bloody fluid in stomach and intestine.
	2000	4/5	Day 2: Impacted stomach, liver patchy, some bloody fluid in stomach and intestine.
	3000	5/5	Day 1 (4) and Day 2 (1): Impacted stomach, liver patchy, some bloody fluid in stomach and intestine.



LD<sub>50</sub> REPORTING FORM USING LITCHFIELD-WILCOXON METHOD

DOSE EFFECT CURVE FOR FOA COMPOUND 71-43 TALC

DOSE	PROPORTION	OBSERVED PERCENT	EXPECTED PERCENT	OBS-EXPCD PERCENT	CONTRIB. TO (CHI) <sup>2</sup>
100	0/5	0	0		
500	1/5	20	20		
1000	3/5	60	56		
2000	4/5	80	88		
3000	5/5	100	96		

Total animals = 25

Number Doses, K = 5

Animals/Dose = 5

(CHI)<sup>2</sup> for n of k-2 = 7.81

Total =                     

(CHI)<sup>2</sup> = .523

Degrees of Freedom, n=k-2 = 3

since .523 is less than 7.81, therefore data not significantly heterogeneous

LD<sub>84</sub> = 1800

LD<sub>50</sub> = 920

LD<sub>16</sub> = 470

$fLD_{50} = s \frac{2.77}{\sqrt{N!}} = \frac{1.958}{2.77} = \frac{1.958}{\sqrt{10}} = (1.958)^{.876} = 1.80$

LD<sub>50</sub> × fLD<sub>50</sub> = (920)(1.80) = 1656

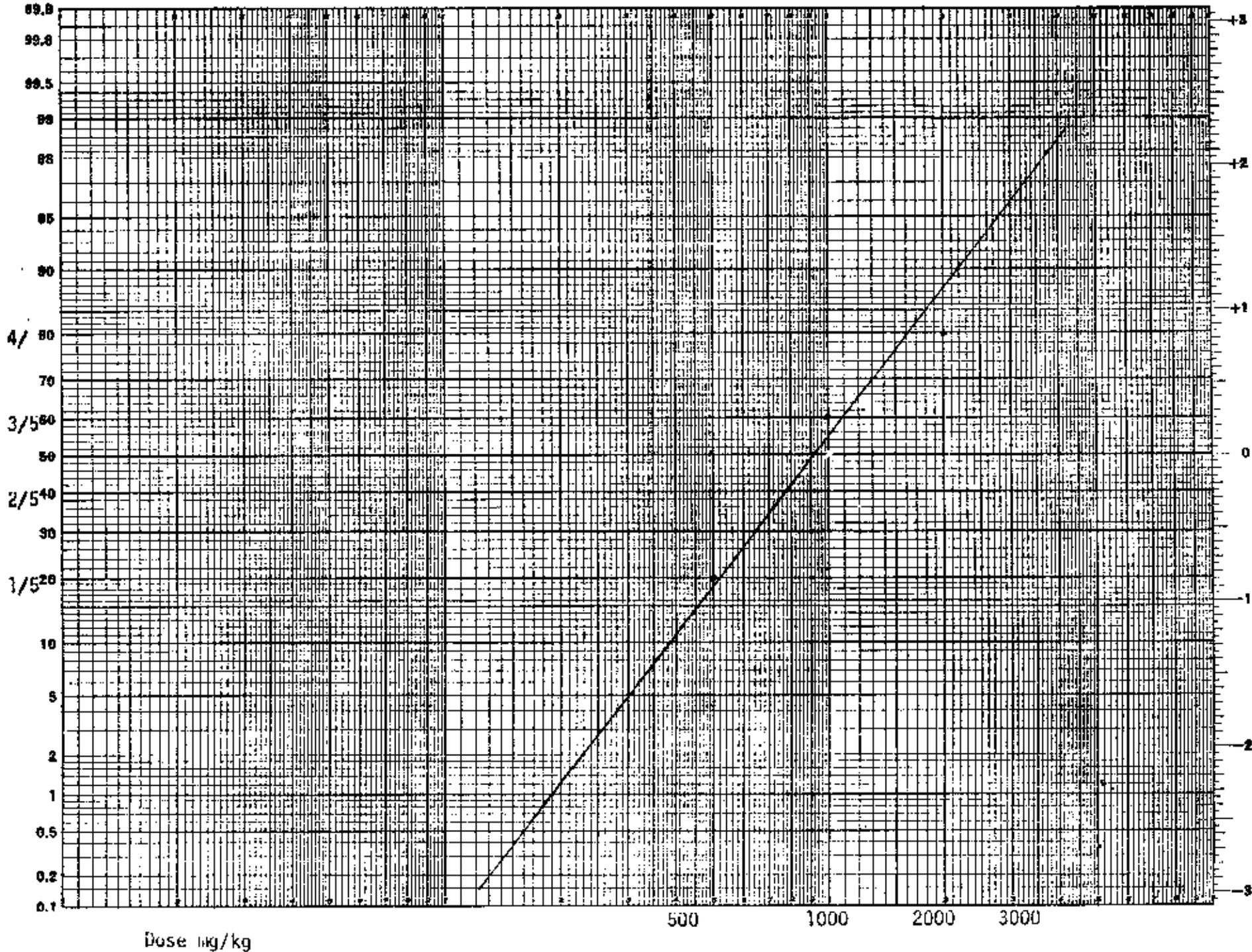
LD<sub>50</sub> = (920)/(1.80) = 511

fLD<sub>50</sub>

LD<sub>50</sub> and 19/20 Confidence Limits = Pr 511 × LD<sub>50</sub> = 1656 = .95

Attached should be a plot of the dose-effect curve on log-probit paper.

01 # Animals Dead/# Animals Used



Compound FDA 71-43 Ta1c

2. Host-Mediated Assay - Test I

Compound FDA 71-43 caused no significant increases in mutant or recombinant frequencies with Salmonella TA-1530 and G-46 or Saccharomyces D3, respectively at the dose levels tested. The in vitro tests were negative.



EVALUATION SHEET

Compound: 71-43 TaIc

Indicator Strain	In Vitro	In Vivo		
		Possible Low Recoveries	Controls	Other Comments
TA-1530 NC, PC, Acutes (7/24/72)	pos.	NC PC AL AI	NC OK PC OK	1. All doses negative
SANC, S-acutes (11/1/72)	(neg.)	AH SANC SAL SAI SAH	SANC OK	
G-46 NC, PC, Acutes (8/7/72)	pos.	NC PC AL AI	NC OK PC OK	1. All doses negative
SANC, S-acutes (8/11/72)	(neg.)	AH SANC SAL SAI SAH	SANC OK	
03 NC, PC, Acutes (7/10/72)	pos.	NC PC AL AI	NC OK PC OK	1. All acute doses negative 2. Subacute doses show slight increase with dose but not even a two fold increase. Would probably call negative without further tests.
SANC, S-acutes (7/14/72)	(neg.)	AH SANC SAL SAI SAH	SANC OK	

Summary: All results from this compound should be accepted. Compound shows no significant activity in vitro or in vivo. The recovery ranges reported in this test are good ones to shoot for- they generally good clear-cut and consistent results.

*David Brink*

a. HOST-MEDIATED ASSAY SUMMARY SHEETS

CONTRACT FDA 71-268

COMPOUND FDA 71-43

TALC

TEST I



HOST MEDIATED ASSAY

SUMMARY SHEET

COMPOUND: FDA 71-43

	SALMONELLA				SACCHAROMYCES D-3	
	TA1530		G-46		MRF (X 10E-5)	MRT/MRC
	MMF (X 10E-8)	MFT/MFC	MMF (X 10E-8)	MFT/MFC		
<b>ACUTE</b>						
NC	.61		.80		5.22	
PC	7.43	12.18	17.67	22.09	59.66	11.43
AL	1.63	2.67	1.85	2.31	2.86	.55
AI	1.58	2.59	1.24	1.55	4.92	.94
ALD5	.92	1.51	1.40	1.75	5.35	1.02
<b>SUBACUTE</b>						
NC	.83		.54		5.75	
SL	2.07	2.49	.91	1.69	4.17	.73
SI	.77	.93	.67	1.24	6.32	1.10
SLO5	1.09	1.31	1.72	3.19	8.59	1.49
PC	8.00		-----		-----	
<b>IN VITRO</b>						
	TA1530	G-46	% CONC	D-3 % SURVIVAL	R X 10E5	
TCPD	-	-	4.0	58.7	2	
NC	-	-	-	100.0	5	
PC	+	+	0.5	68.8	267	

STCP

**b. HOST-MEDIATED ASSAY DATA SHEETS**

**CONTRACT FDA 71-268**

**COMPOUND FDA 71-43**

**TALC**

**TEST I**



## Host Mediated Assay - Adjusted Raw CFU x $10^7/0.6$ ml

The true raw colony counts were lost through automation for this compound. Thus, the source of the adjusted raw CFU x  $10^7/0.6$  ml (Column A) was the true raw counts as assimilated by the automatic colony counter, multiplied by the automatic program by 0.16666666666667 (Column B) and then divided by 0.1667 (the check figure). The original concept was that the true CFU x  $10^7/0.6$  ml would be printed as column A. Through a programming anomaly the Column B check figure was obtained as the raw CFU x  $10^7/0.6$  ml and recorded as such.

- Step 1: Technician set counter - plates on counter.
- Step 2: Automatic equipment accumulates counts on 3 plates of  $10^{-6}$  dilution as CFU x  $10^7/0.6$  ml.
- Step 3: Automatic equipment multiplies count obtained in step 1 by 0.16666666666667 to obtain total count/ml at  $10^8$ .
- Step 4: Automatic check of result of step 3.  
 $TC \times 10^8 \div 0.1667 = CFU \times 10^7/0.6$  ml
- Step 5: Technician was to record the true raw CFU x  $10^7/0.6$  ml in log book, however, the computer developed a quirk and provided the Column B check figure as the raw count.

To clarify the problem Column A is headed Adjusted Raw CFU X  $10E^7/0.6$  ml in each case where the check figure was provided as the raw count.



HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA 7A1530

DOSE LEVEL: NEGATIVE CONTROL - SALINE (ACUTE TRIALS)

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 24, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	28.50	4.75	3.00	.63
2	56.94	9.49	1.00	.11
3	18.42	3.07	4.00	1.30 *
4	55.98	9.33	2.00	.21
5	21.72	3.62	2.00	.55
6	38.70	6.45	3.00	.47
7	14.10	2.35	1.00	.43
8	29.22	4.87	4.00	.82
9	22.56	3.76	3.00	.80
10	32.25	5.38	4.00	.74

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.31	2.70	.61
RANGE	7.14	3.00	1.20
MAX	9.49	4.00	1.30
MIN	2.35	1.00	.11

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.56	2.56	.53
RANGE	7.14	3.00	.72
MAX	9.49	4.00	.82
MIN	2.35	1.00	.11

TOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: POSITIVE CONTROL - DMN - 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 24, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	20.28	3.38	29.00	8.58
2	19.74	3.29	24.00	7.29
3	88.18	14.70	26.00	1.77
4	21.18	3.53	23.00	6.52
5	19.50	3.25	38.00	11.69
6	17.22	2.87	18.00	6.27
7	19.14	3.19	24.00	7.52
8	12.36	2.06	21.00	10.19
9	16.98	2.33	26.00	9.19
10	32.88	5.48	29.00	5.29

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.46	25.80	7.43
RANGE	12.64	20.00	9.92
MAX	14.70	38.00	11.69
MIN	2.06	18.00	1.77

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	3.32	25.78	8.06
RANGE	3.42	20.00	6.40
MAX	5.48	38.00	11.69
MIN	2.06	18.00	5.29

TOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: LOW - 30 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 24, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	11.34	1.89	6.00	3.17
2	13.14	2.19	1.00	.46
3	16.56	2.76	4.00	1.45
4	17.88	2.98	8.00	2.68
5	20.94	3.49	6.00	1.72
6	46.14	7.69	5.00	.65
7	18.48	3.08	4.00	1.30

NO. OF ANIMALS EQUALS 7  
NO. OF CONTAMINATED EQUALS 3

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	3.44	4.86	1.63
RANGE	5.60	7.00	2.72
MAX	7.69	8.00	3.17
MIN	1.89	1.00	.46

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA T61530.

DOSE LEVEL: INTERMEDIATE - 300 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 24, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	14.34	2.39	3.00	1.26
2	14.52	2.42	2.00	.83
3	13.92	2.32	7.00	3.02
4	40.98	6.83	10.00	1.46
5	27.90	4.65	4.00	.86
6	14.23	2.38	3.00	1.26
7	40.74	6.79	7.00	1.03
8	20.52	3.42	9.00	2.63
9	13.14	2.19	5.00	2.28
10	20.22	3.37	4.00	1.19

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	3.68	5.40	1.58
RANGE	4.64	8.00	2.19
MAX	6.83	10.00	3.02
MIN	2.19	2.00	.83

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: LD5 - 3000 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 24, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	45.42	7.57	9.00	1.19
2	12.54	2.09	3.00	1.44
3	11.94	1.99	2.00	1.01
4	13.68	2.28	1.00	.44
5	48.48	8.08	8.00	.99
6	10.74	1.79	2.00	1.12
7	59.34	9.89	7.00	.71
8	59.94	9.99	2.00	.20
9	50.58	8.43	9.00	1.07
10	22.56	3.75	4.00	1.06

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.59	4.70	.92
RANGE	8.20	8.00	1.24
MAX	9.99	9.00	1.44
MIN	1.79	1.00	.20

NO. OUTLIERS

DP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1538

DOSE LEVEL: NEGATIVE CONTROL - SUBACUTE TRIALS

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: NOVEMBER 1, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.5ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	8.70	1.45	3.00	2.07 *
2	16.40	2.73	2.00	.73
3	17.80	2.97	1.00	.34
4	16.80	2.80	1.00	.36
5	12.80	2.13	1.00	.47
6	13.40	2.23	2.00	.90
7	15.20	2.70	2.00	.74
8	22.70	3.78	4.00	1.06

NO. OF ANIMALS EQUALS 8  
 TOTAL CFU OUT OF RANGE EQUALS 1  
 SAMPLES WITH ZERO MUTANTS EQUAL 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	2.60	2.00	.63
RANGE	2.33	3.00	1.73
MAX	3.78	4.00	2.07
MIN	1.45	1.00	.34

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	2.76	1.86	.66
RANGE	1.65	3.00	.72
MAX	3.78	4.00	1.06
MIN	2.13	1.00	.34

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1538

DOSE LEVEL: POSITIVE CONTROL - DMN - 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: NOVEMBER 1, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	35.20	5.87	38.00	6.48
2	22.80	3.80	42.00	11.05
3	31.70	5.28	28.00	5.30
4	19.20	3.20	30.00	9.37
5	28.40	4.73	22.00	4.65
6	31.00	5.17	36.00	6.97
7	23.10	3.85	47.00	12.21

NO. OF ANIMALS EQUALS 7  
 NO. OF CONTAMINATED EQUALS 1  
 TOTAL CFU OUT OF RANGE EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.56	34.71	8.00
RANGE	2.67	25.00	7.56
MAX	5.87	47.00	12.21
MIN	3.20	22.00	4.65

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1538

DOSE LEVEL: LOW - 30 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: NOVEMBER 1, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	6.90	1.15	3.00	2.61
2	17.70	2.95	4.00	1.36
3	17.50	2.92	2.00	.69
4	8.50	1.42	1.00	.71
5	16.50	2.75	3.00	1.09
6	14.00	2.33	2.00	.86
7	10.80	1.80	8.00	4.44
8	17.40	2.90	14.00	4.83

NO. OF ANIMALS EQUALS 8  
NO. OF CONTAMINATED EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	2.28	4.63	2.07
RANGE	1.80	13.00	4.14
MAX	2.95	14.00	4.83
MIN	1.15	1.00	.69

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: INTERMEDIATE - 300 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: NOVEMBER 1, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	41.40	6.90	4.00	.58
2	27.70	4.62	3.00	.65
3	33.50	5.58	5.00	.90
4	32.20	5.37	8.00	1.49
5	22.80	3.80	1.00	.26
6	70.60	11.77	7.00	.59
7	27.30	4.55	2.00	.44
8	29.10	4.85	6.00	1.24

NO. OF ANIMALS EQUALS 8  
 NO. OF CONTAMINATED EQUALS 1  
 TOTAL CFU OUT OF RANGE EQUALS 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.93	4.50	.77
RANGE	7.97	7.00	1.23
MAX	11.77	8.00	1.49
MIN	3.80	1.00	.26

NO OUTLIERS

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA YA1536

DOSE LEVEL: LD5 - 3000 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: NOVEMBER 1, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	51.70	8.62	11.00	1.28
2	20.80	3.47	3.00	.87
3	21.70	3.62	2.00	.55
4	34.00	5.67	4.00	.71
5	12.70	2.12	2.00	.94
6	24.30	4.05	6.00	1.48
7	8.80	1.47	3.00	2.05
8	35.30	5.83	5.00	.85

NO. OF ANIMALS EQUALS 8  
TOTAL CFU OUT OF RANGE EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.36	4.50	1.09
RANGE	7.15	9.00	1.49
MAX	8.62	11.00	2.05
MIN	1.47	2.00	.55

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.77	4.71	.95
RANGE	6.50	9.00	.93
MAX	8.62	11.00	1.48
MIN	2.12	2.00	.55

10021 E ILLS I-FORMAT INPUT  
 10077 INS  
 10022 LIST ITEM AT 100154. INPUT FIELD IS <??>  
 ALABACK SEQUENCE  
 10077 ENTRY LINE ADDRESS CALLER LINE ADDRESS  
 10077 007317 155. 001024

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA 9-46

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: AUGUST 7, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE' (C/B) X 10E-8
1	43.86	7.31	4.00	.55
2	45.90	7.65	6.00	.78
3	49.38	8.23	7.00	.85
4	33.78	5.63	3.00	.53
5	46.02	7.67	4.00	.52
6	28.82	4.80	4.00	.83
7	29.58	4.93	7.00	1.42
8	25.68	4.28	3.00	.70
9	29.34	4.89	5.00	1.02

NO. OF ANIMALS EQUALS 9  
 SAMPLES WITH ZERO MUTANTS EQUAL 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	6.15	4.78	.80
RANGE	3.95	4.00	.90
MAX	8.23	7.00	1.42
MIN	4.28	3.00	.52

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	6.31	4.50	.72
RANGE	3.95	4.00	.50
MAX	8.23	7.00	1.02
MIN	4.28	3.00	.52

TOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: POSITIVE CONTROL - DMN - 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: AUGUST 7, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE. (C/B) X 10E-8
1	39.48	6.58	84.00	12.77
2	38.58	6.43	118.00	18.35
3	25.50	4.25	48.00	11.29
4	38.10	6.35	78.00	12.28
5	47.34	7.89	108.00	13.69
6	25.33	4.23	97.00	22.93
7	25.26	4.21	49.00	11.64
8	23.64	3.94	117.00	29.69
9	53.02	9.67	92.00	9.51
10	15.33	2.55	88.00	34.51

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.61	87.90	17.67
RANGE	7.12	70.00	25.00
MAX	9.67	118.00	34.51
MIN	2.55	48.00	9.51

NO OUTLIERS

OP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: LOW - 30 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: AUGUST 7, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	12.18	2.03	4.00	1.97
2	24.54	4.09	3.00	.73
3	38.28	6.38	2.00	.31
4	12.18	2.03	12.00	5.91 *
5	41.28	6.86	14.00	2.03
6	47.94	7.99	5.00	.63
7	30.90	5.15	6.00	1.17
8	29.58	4.93	10.00	2.03

NO. OF ANIMALS EQUALS 8  
TOTAL CFU OUT OF RANGE EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.94	7.00	1.85
RANGE	5.96	12.00	5.60
MAX	7.99	14.00	5.91
MIN	2.03	2.00	.31

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.35	6.29	1.27
RANGE	5.96	12.00	1.72
MAX	7.99	14.00	2.03
MIN	2.03	2.00	.31

OP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: INTERMEDIATE - 300 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: AUGUST 7, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	23.88	3.98	5.00	1.26
2	34.32	5.72	9.00	1.57
3	30.72	5.12	3.00	.59
4	27.12	4.52	6.00	1.33
5	30.18	5.03	2.00	.40
6	34.44	5.74	7.00	1.22
7	20.10	3.35	3.00	.90
8	41.70	6.95	4.00	.58
9	29.82	4.97	4.00	.80
10	27.12	4.52	17.00	3.76 *

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.99	6.00	1.24
RANGE	3.50	15.00	3.36
MAX	6.95	17.00	3.76
MIN	3.35	2.00	.40

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.04	4.78	.56
RANGE	3.50	7.00	1.18
MAX	6.95	9.00	1.57
MIN	3.35	2.00	.40

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G#46

DOSE LEVEL: LD5 - 3000 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: AUGUST 7, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	17.50	2.92	3.00	1.03
2	55.14	9.19	4.00	.44
3	31.14	5.19	1.00	.19
4	45.42	7.57	8.00	1.06
5	61.68	10.28	3.00	.29
6	43.68	7.28	1.00	.14
7	58.68	9.78	5.00	.51
8	18.30	3.05	23.00	7.54 *

NO. OF ANIMALS EQUALS 8  
TOTAL CFU OUT OF RANGE EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	6.91	6.00	1.40
RANGE	7.36	22.00	7.40
MAX	10.28	23.00	7.54
MIN	2.92	1.00	.14

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	7.46	3.57	.52
RANGE	7.36	7.00	.92
MAX	10.28	8.00	1.06
MIN	2.92	1.00	.14

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA 6-46

DOSE LEVEL: NEGATIVE CONTROL - SALINE (SUBACUTE TRIALS)

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: AUGUST 11, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	28.50	4.75	4.00	.84
2	42.06	7.01	4.00	.57
3	20.34	3.39	2.00	.59
4	45.66	7.61	6.00	.79
5	32.76	5.46	1.00	.18
6	29.94	4.99	3.00	.60
7	34.44	5.74	2.00	.35
8	21.06	3.51	2.00	.57
9	23.58	3.93	3.00	.76
10	31.86	5.31	1.00	.19

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	5.17	2.80	.54
RANGE	4.22	5.00	.66
MAX	7.61	6.00	.84
MIN	3.39	1.00	.18

NO OUTLIERS

TOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G<sup>-</sup>46

DOSE LEVEL: LOW -30 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: AUGUST 11, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	12.30	2.05	5.00	2.44
2	25.50	4.25	1.00	.24
3	54.42	9.07	2.00	.22
4	38.52	6.42	1.00	.16
5	13.14	2.19	4.00	1.83
6	21.12	3.52	2.00	.57
7	51.18	8.53	5.00	.59
8	13.98	2.33	3.00	1.29

NO. OF ANIMALS EQUALS B  
 NO. OF CONTAMINATED EQUALS 1  
 TOTAL CFU OUT OF RANGE EQUALS 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	4.80	2.88	.91
RANGE	7.02	4.00	2.28
MAX	9.07	5.00	2.44
MIN	2.05	1.00	.16

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: INTERMEDIATE - 300 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: AUGUST 11, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	53.94	8.99	3.00	.33
2	33.54	5.59	1.00	.18
3	44.70	7.45	4.00	.54
4	41.70	6.95	4.00	.58
5	56.28	9.38	5.00	.53
6	23.82	3.97	3.00	.76
7	10.50	1.75	3.00	1.71 *
8	43.82	7.17	5.00	.70
9	41.88	6.98	4.00	.57
10	15.90	2.65	2.00	.75

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	6.09	3.40	.67
RANGE	7.63	4.00	1.54
MAX	9.38	5.00	1.71
MIN	1.75	1.00	.18

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	6.57	3.44	.55
RANGE	6.73	4.00	.58
MAX	9.38	5.00	.76
MIN	2.65	1.00	.18

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G#46

DOSE LEVEL: LD5 - 3000 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: AUGUST 11, 1972

ANIMAL NUMBER	A ADJUSTED RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	15.12	2.52	3.00	1.19
2	32.58	5.43	8.00	1.47
3	11.28	1.88	3.00	1.60
4	23.82	3.97	4.00	1.01
5	10.50	1.75	7.00	4.00 *
6	7.74	1.29	3.00	2.33
7	9.54	1.59	2.00	1.26
8	13.02	2.17	2.00	.92

NO. OF ANIMALS EQUALS 8  
TOTAL CFU OUT OF RANGE EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	2.58	4.00	1.72
RANGE	4.14	6.00	3.08
MAX	5.43	8.00	4.00
MIN	1.29	2.00	.92

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	2.69	3.57	1.40
RANGE	4.14	6.00	1.40
MAX	5.43	8.00	2.33
MIN	1.29	2.00	.92

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 10, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	415.00	.42	2.00	4.82
2	240.00	.24	1.00	4.17
3	493.00	.49	3.00	6.09
4	271.00	.27	1.00	3.69
5	436.00	.44	2.00	4.59
6	218.00	.22	1.00	4.59
7	253.00	.25	2.00	7.91
8	440.00	.44	3.00	6.82
9	287.00	.29	1.00	3.48
10	397.00	.40	2.00	5.04
TOTAL		3.45	18.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 5.22

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.34	1.80	5.12
RANGE	.28	2.00	4.42
MAX	.49	3.00	7.91
MIN	.22	1.00	3.48

NO. OUTLIERS

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: POSITIVE CONTROL - EMS - 350 MG/KG I.M.

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 10, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	279.00	.28	10.00	35.84
2	368.00	.37	14.00	38.04
3	217.00	.22	14.00	64.52
4	248.00	.25	36.00	145.16
5	723.00	.72	47.00	65.01
6	253.00	.25	13.00	51.38
7	411.00	.41	18.00	43.80
8	490.00	.49	26.00	53.06
9	246.00	.25	15.00	60.98
TOTAL		3.23	193.00	

NO. OF ANIMALS EQUALS 9

NO. OF DEAD ANIMALS EQUALS 1

MEAN C/MEAN B = 59.66

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.36	21.44	61.98
RANGE	.51	37.00	109.32
MAX	.72	47.00	145.16
MIN	.22	10.00	35.84

\* SUMMARY WITH OUTLIERS REMOVED

MEAN C/MEAN B = 52.56

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.37	19.63	51.58
RANGE	.51	37.00	29.16
MAX	.72	47.00	65.01
MIN	.22	10.00	35.84

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3.

DOSE LEVEL: LOW - 30 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 10, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	227.00	.23	1.00	4.41
2	209.00	.21	0.	0.
3	317.00	.32	1.00	3.15
4	406.00	.41	1.00	2.46
5	273.00	.27	1.00	3.66
6	472.00	.47	2.00	4.24
7	549.00	.55	1.00	1.82
8	433.00	.40	2.00	4.96
9	289.00	.29	0.	0.
TOTAL		3.14	9.00	

NO. OF ANIMALS EQUALS 9

NO. OF DEAD ANIMALS EQUALS 1

MEAN C/MEAN B = 2.86

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.35	1.00	2.75
RANGE	.34	2.00	4.96
MAX	.55	2.00	4.96
MIN	.21	0.	0.

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: INTERMEDIATE - 300 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 10, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	209.00	.21	1.00	4.78
2	413.00	.41	2.00	4.84
3	492.00	.49	1.00	2.03
4	407.00	.41	2.00	4.91
5	390.00	.39	2.00	5.13
6	482.00	.48	3.00	6.22
7	302.00	.30	1.00	3.31
8	337.00	.34	3.00	8.90
9	422.00	.42	2.00	4.74
TOTAL		3.45	17.00	

NO. OF ANIMALS EQUALS 9  
NO. OF CONTAMINATED EQUALS 1

MEAN C/MEAN B = 4.92

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.38	1.89	4.99
RANGE	.28	2.00	6.87
MAX	.49	3.00	8.90
MIN	.21	1.00	2.03

\* SUMMARY WITH OUTLIERS REMOVED

MEAN C/MEAN B = 4.49

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.39	1.75	4.50
RANGE	.28	2.00	4.19
MAX	.49	3.00	6.22
MIN	.21	1.00	2.03

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: LD5 - 3000 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 10, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	501.00	.50	4.00	7.98
2	417.00	.42	3.00	7.19
3	641.00	.64	3.00	4.68
4	411.00	.41	1.00	2.43
5	299.00	.21	0.	0.
6	414.00	.41	2.00	4.83
7	217.00	.22	2.00	9.22
8	663.00	.66	3.00	4.52
9	415.00	.42	3.00	7.23
10	412.00	.41	2.00	4.85

TOTAL 4.30 23.00

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 5.35

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.43	2.30	5.29
RANGE	.45	4.00	9.22
MAX	.66	4.00	9.22
MIN	.21	0.	0.

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: NEGATIVE CONTROL - SALINE (SUBACUTE TRIALS)

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JULY 14, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	451.00	.45	2.00	4.43
2	492.00	.49	3.00	6.10
3	283.00	.28	1.00	3.53
4	376.00	.38	2.00	5.32
5	241.00	.24	1.00	4.15
6	297.00	.30	1.00	3.37
7	381.00	.38	3.00	7.87
8	366.00	.37	2.00	5.46
9	291.00	.29	2.00	6.87
10	472.00	.47	4.00	8.47
TOTAL		3.65	21.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 5.75

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.36	2.10	5.56
RANGE	.25	3.00	5.11
MAX	.49	4.00	8.47
MIN	.24	1.00	3.37

NO OUTLIERS

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: LOW = 30 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: JULY 14, 1972

ANIMAL NUMBER	A RAW CFU X 10 <sup>5</sup> /1.0ML	B TOTAL CFU SCREENED X 10 <sup>5</sup> /1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10 <sup>-5</sup>
1	317.00	.32	1.00	3.15
2	322.00	.32	1.00	3.11
3	321.00	.32	2.00	6.23
4	509.00	.51	2.00	3.93
5	243.00	.24	1.00	4.12
6	602.00	.60	3.00	4.98
7	317.00	.32	2.00	6.31
8	483.00	.48	2.00	4.14
9	311.00	.31	1.00	3.22
10	419.00	.41	1.00	2.44
TOTAL		3.83	16.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 4.17

	COL. B (X 10 <sup>5</sup> )	COL. C (X 10 <sup>0</sup> )	COL. D (X 10 <sup>-5</sup> )
MEAN	.38	1.60	4.16
RANGE	.36	2.00	3.87
MAX	.60	3.00	6.31
MIN	.24	1.00	2.44

NO. OUTLIERS

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: INTERMEDIATE - 300 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: JULY 14, 1972

ANIMAL NUMBER	A RA# CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	452.00	.45	3.00	6.64
2	459.00	.46	4.00	8.71
3	434.00	.43	4.00	9.22
4	602.00	.60	5.00	8.31
5	421.00	.42	2.00	4.75
6	418.00	.42	1.00	2.39
7	328.00	.33	3.00	9.15
8	492.00	.49	2.00	4.07
9	283.00	.29	1.00	3.50
10	219.00	.22	1.00	4.57

TOTAL 4.11 26.00

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 6.32

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.41	2.60	6.13
RANGE	.38	4.00	6.82
MAX	.60	5.00	9.22
MIN	.22	1.00	2.39

NO OUTLIERS

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3.

DOSE LEVEL: LDB - 3000 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: JULY 14, 1972

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	413.00	.41	4.00	9.69
2	287.00	.29	2.00	6.97
3	412.00	.41	5.00	12.14 *
4	268.00	.27	1.00	3.73 *
5	413.00	.41	3.00	7.26
6	419.00	.42	3.00	7.16
7	247.00	.25	2.00	8.10
8	622.00	.62	6.00	9.65
9	528.00	.53	5.00	9.47
TOTAL		3.61	31.00	

NO. OF ANIMALS EQUALS 9  
NO. OF CONTAMINATED EQUALS 1

MEAN C/MEAN B = 8.59

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.40	3.44	8.24
RANGE	.37	5.00	8.40
MAX	.62	6.00	12.14
MIN	.25	1.00	3.73

\* SUMMARY WITH OUTLIERS REMOVED

MEAN C/MEAN B = 8.54

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.42	3.57	8.33
RANGE	.37	4.00	2.72
MAX	.62	6.00	9.69
MIN	.25	2.00	6.97

STOP

### 3. Toxicity Data - Test II

#### a. Acute toxicity test

Compound FDA 71-43 was prepared as a 18.3% (w/v) suspension in saline and administered orally to a group of ten male rats (average body weight 219.2 grams) at a single dose of 5000 mg/kg.

No signs of toxicity or abnormal behavior were observed in the seven-day observation period. No deaths occurred. At termination all animals were killed and on necropsy no gross findings were observed.

The acute oral  $LD_{50}$  for compound FDA 71-43 is considered to be greater than 5000 mg/kg.

#### b. Subacute toxicity test

Compound FDA 71-43 was prepared as a suspension (29.6% [w/v]) in saline and administered orally, daily for five days at a dosage level of 5000 mg/kg per day. The test substance was given to a group of five animals (average body weight 367.0 grams) which showed minimal signs of toxicity consisting of slightly rough fur, decreased activity and light colored stools (presumably due to coloration of the compound). No gross pathologic evidence was observed at necropsy.

The 14-day subacute oral  $LD_{50}$  for compound FDA 71-43 is considered to be greater than 5 grams per kilogram.



c. TOXICITY DATA SHEETS  
CONTRACT FDA 71-268  
COMPOUND FDA 71-43  
TALC  
TEST II



ACUTE  
TOXICITY DATA  
CONTRACT FDA 71-268  
COMPOUND FDA 71-43  
TALC

Solvent: 0.85% saline  
Dosage Form: Suspension  
Animals: Male rats with an average body weight of 219.2 grams.  
All animals were observed for seven (7) days.  
LD<sub>50</sub>: Could not be determined at a dosage of 5 grams per kilogram. The LD<sub>50</sub> is greater than 5 grams per kilogram and there was no abnormal gross pathology in the animals used in this study.



SUBACUTE  
TOXICITY DATA  
CONTRACT FDA 71-268  
COMPOUND FDA 71-43  
TALC

Solvent: 0.85%  
Dosage Form: Suspension  
Animals: Male rats with an average body weight of 367.0 grams.  
All animals were observed for 14 days.

<u>Dose</u> <u>mg/kg</u>	<u># Dead</u> <u># Animals</u>	<u>Signs of Toxicity</u>
5000	0/10	Slight signs of rough fur, reduced activity and pale appearing feces.

LD<sub>50</sub>: Could not be determined at a dosage of 5 grams per kilogram per day. The LD<sub>50</sub> is greater than 5 grams per kilogram per day. There were no gross signs of pathologic alteration in the animals used in this study.



4. Host-Mediated Assay - Test II

The new dose evaluation on compound FDA 71-43, Talc, was not active in the tests against Salmonella strains TA-1530 and G-46 or the Saccharomyces strain D3 when tested acutely or subacutely at a 5000 mg/kg dose level.

  
David Brusick



a. **HOST-MEDIATED ASSAY SUMMARY SHEETS**

**CONTRACT FDA 71-268**

**COMPOUND FDA 71-43**

**TALC**

**TEST II**



HOST MEDIATED ASSAY

SUMMARY SHEET

COMPOUND: FDA 71-43

	SALMONELLA		SALMONELLA		SACCHAROMYCES D-3	
	TA1530	G-46	TA1530	G-46	TA1530	G-46
	MMF (X 10E-8)	MFT/MFC	MMF (X 10E-8)	MFT/MFC	MRF (X 10E-5)	MRT/MRC
<b>ACUTE</b>						
NC	2.38		.96		15.63	
PC	32.74	13.76	80.22	83.56	132.42	8.47
AL	0.	0.	0.	0.	0.	0.
AI	0.	0.	0.	0.	0.	0.
AH	2.61	1.10	2.76	2.88	13.90	.89
<b>SUBACUTE</b>						
NC	1.00		1.00		1.00	
SL	0.	0.	0.	0.	0.	0.
SI	0.	0.	0.	0.	0.	0.
SH	0.	0.	0.	0.	0.	0.
<b>IN VITRO</b>						
	TA1530	G-46		D-3		
			% CONC	% SURVIVAL	R X 10E5	
NC						
PC						

STOP  
SRU'S: .4

ST

HOST MEDIATED ASSAY

SUMMARY SHEET

COMPOUND: FDA 71-43

	SALMONELLA				SACCHAROMYCES D-3	
	TA1530		G-46		MRF	MRT/MRC
	MMF (X 10E-8)	MFT/MFC	MMF (X 10E-8)	MFT/MFC	(X 10E-5)	
<b>ACUTE</b>						
NC	1.64		1.00		16.05	
PC	72.14	43.99	82.84	82.84	83.24	5.19
AL	0.	0.	0.	0.	0.	0.
AI	0.	0.	0.	0.	0.	0.
AH	0.	0.	0.	0.	0.	0.
<b>SUBACUTE</b>						
NC	1.64		1.00		16.05	
SL	0.	0.	0.	0.	0.	0.
SI	0.	0.	0.	0.	0.	0.
SH	1.55	.95	.98	.98	19.42	1.21
<b>IN VITRO</b>						
	TA1530	G-46	% CONC	% SURVIVAL	D-3	R X 10E5
NC						
PC						

STOP  
SRU'S: .4

b. HOST-MEDIATED ASSAY DATA SHEETS

CONTRACT FDA 71-268

COMPOUND FDA 71-43

TALC

TEST II



**HOST MEDIATED ASSAY REPORT SHEET**

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1538

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 20, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	57.70	9.62	28.00	2.91
2	75.90	12.65	32.00	2.53
3	68.80	14.80	35.00	2.36
4	47.70	7.95	23.00	2.89
5	49.60	8.27	25.00	3.02
6	69.00	11.50	24.00	2.09
7	57.60	9.60	12.00	1.25
8	72.10	12.02	27.00	2.25
9	74.50	12.42	22.00	1.77
10	65.70	10.95	30.00	2.74

NO. OF ANIMALS EQUALS: 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	10.98	25.80	2.38
RANGE	6.85	23.00	1.77
MAX	14.80	35.00	3.02
MIN	7.95	12.00	1.25

NO OUTLIERS

GROUP

**HOST MEDIATED ASSAY REPORT SHEET**

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1538

DOSE LEVEL: POSITIVE CONTROL -- DMN -- 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 20, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	65.30	10.88	255.00	23.43
2	38.00	6.33	142.00	22.42
3	67.70	11.28	281.00	24.90
4	57.20	9.53	372.00	39.02
5	58.80	9.80	357.00	36.43
6	50.20	8.37	109.00	13.03
7	75.80	12.63	690.00	54.62
8	53.80	8.97	332.00	37.03
9	62.90	10.48	267.00	25.47
10	75.00	12.50	638.00	51.04

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	10.08	344.30	32.74
RANGE	6.33	581.00	41.59
MAX	12.63	690.00	54.62
MIN	6.33	109.00	13.03

NO OUTLIERS

STOP

# HOST-MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1538

DOSE LEVEL: HIGH -- 5000 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 20, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE. (C/B) X 10E-8
1	97.40	16.23	37.00	2.28
2	74.40	12.40	25.00	2.02
3	104.60	17.43	48.00	2.75
4	60.90	10.15	35.00	3.46
5	102.90	17.15	28.00	1.63
6	58.30	9.72	33.00	3.40
7	85.00	14.17	36.00	2.54
8	102.90	17.15	48.00	2.80

NO. OF ANIMALS EQUALS: 8  
 TOTAL CFU OUT OF RANGE EQUALS: 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	14.30	36.25	2.61
RANGE	7.72	25.00	1.82
MAX	17.43	48.00	3.46
MIN	9.72	25.00	1.63

NO OUTLIERS

STOP  
 END OF 14 W. INS. EOF  
 ALKBACK SEQUENCE  
 PROGRAM ENTRY LINE ADDRESS CALLER LINE ADDRESS  
 \$F10 \$F10 007317 155 001024

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: FEBRUARY 22, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	86.80	14.47	51.00	3.53
2	112.30	18.72	37.00	1.98
3	118.00	19.67	19.00	.97
4	76.00	12.67	27.00	2.13
5	80.60	13.43	11.00	.82
6	101.60	16.93	24.00	1.42
7	126.40	21.07	31.00	1.47
8	51.50	8.58	15.00	1.75
9	84.70	14.12	19.00	1.35
10	156.60	26.10	26.00	1.00

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	16.58	26.00	1.64
RANGE	17.52	40.00	2.71
MAX	26.10	51.00	3.53
MIN	8.58	11.00	.82

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	16.81	23.22	1.43
RANGE	17.52	26.00	1.31
MAX	26.10	37.00	2.13
MIN	8.58	11.00	.82

STOP  
RU'S: .7

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: POSITIVE CONTROL - DMN - 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 22, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	100.00	16.67	600.00	36.00
2	63.10	10.52	1008.00	95.85
3	60.10	10.02	812.00	81.06
4	80.80	13.47	772.00	57.33
5	44.90	7.48	299.00	39.95
6	101.80	16.97	1272.00	74.97
7	69.00	11.50	1635.00	142.17
8	105.60	17.60	1356.00	77.04
9	90.40	15.07	759.00	50.38
10	74.50	12.42	827.00	66.60

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	13.17	934.00	72.14
RANGE	10.12	1336.00	106.17
MAX	17.60	1635.00	142.17
MIN	7.48	299.00	36.00

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	13.36	856.11	64.35
RANGE	10.12	1057.00	59.85
MAX	17.60	1356.00	95.85
MIN	7.48	299.00	36.00

STOP  
SRU'S: .6

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!  
!

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA TA1530

DOSE LEVEL: HIGH - 5000 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: FEBRUARY 22, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.6ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	119.80	19.97	37.00	1.85
2	127.60	21.27	42.00	1.97
3	133.00	22.17	32.00	1.44
4	132.90	22.15	37.00	1.67
5	173.50	28.92	18.00	.62
6	81.70	13.62	15.00	1.10
7	104.10	17.35	44.00	2.54
8	186.50	31.08	38.00	1.22

NO. OF ANIMALS EQUALS 8  
NO. OF CONTAMINATED EQUALS 2

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	22.07	32.88	1.55
RANGE	17.47	29.00	1.91
MAX	31.08	44.00	2.54
MIN	13.62	15.00	.62

NO OUTLIERS

STOP  
SRU'S: .5

HOST-MEDIATED ASSAY REPORT SHEET

COMPOUND: FC-71-43

ORGANISM: SALMONELLA 6-46

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: I.V. VIVO, ORAL, ACUTE

DATE STARTED: JANUARY 30, 1974

ANIMAL NUMBER	A RAW CFU X 10 <sup>27</sup> /0.1ML	B TOTAL CFU X 10 <sup>28</sup> /1.0ML	C TOTAL NO. MUTANTS X 10 <sup>20</sup> /1.0ML	D MUTATION FRE (C/B) X 10 <sup>-8</sup>
1	60.30	10.05	15.00	1.47
2	99.45	16.57	15.00	.91
3	95.50	15.12	15.00	.90
4	71.3	11.83	16.00	1.35
5	95.90	15.9	11.00	.69
6	89.0	14.58	10.00	.67
7	52.1	8.68	7.00	.81
8	64.20	10.70	13.00	1.21
9	104.00	17.48	10.00	.57
10	60.20	10.03	10.00	1.00

NO. OF ANIMALS EQUALS 1

	COL. B (X 10 <sup>28</sup> )	COL. C (X 10 <sup>20</sup> )	COL. D (X 10 <sup>-8</sup> )
MEAN	13.22	12.20	.90
RANGE	3.50	9.00	.92
SD	17.48	16.00	1.49
MIN	8.68	7.00	.57

NO OUTLIERS

ICP

POST ASSOCIATED ASSAY REPORT SHEET

COMPOUND: FD: 71-43

ORGANISM: SALMONELLA 6-46

DOSE LEVEL: POSITIVE CONTROL - DMN - 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JANUARY 30, 1974

ANIMAL NUMBER	A BAK CFU X 10E7/0.1ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	90.80	15.13	631.00	41.70
2	70.50	11.75	1314.00	111.33
3	74.00	14.00	1462.00	104.43
4	105.50	17.58	751.00	42.71
5	33.90	13.98	1704.00	121.86
6	88.90	14.82	1127.00	76.06
7	82.00	13.23	947.00	68.46
8	53.10	8.85	953.00	96.38
9	87.70	14.62	915.00	62.60
10	90.80	16.55	1231.00	76.19

NO. OF ANIMALS - QUERLS 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	14.11	1096.50	80.22
SD	6.73	1073.00	80.16
MAX	17.58	1704.00	121.86
MIN	8.85	631.00	41.70

NO OUTLIERS

STOP

HOST ADAPTED ASSAY REPORT SHEET

COMPOUND: FO: 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: HIGH - 5000 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: JANUARY 30, 1974

ANIMAL NUMBER	DATA CFU X 10E7/0.5ML	TOTAL CFU X 10E8/1.0ML	TOTAL NO. MUTANTS X 10E0/1.0ML	MUTATION FREQUENCY X 10E-8
1	66.20	11.03	160.00	14.50
2	63.90	10.65	18.00	1.59
3	78.60	12.77	9.00	.70
4	47.50	7.92	9.00	1.14
5	48.90	8.13	14.00	1.72
6	69.00	11.50	4.00	.5
7	39.50	6.42	6.00	.94
8	58.90	9.80	10.00	1.02

NO. OF ANIMALS FOUND

NO. OF CONTAMINATED SPECIMENS

TOTAL CFU OUT OF RANGE EQUALS

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	9.78	28.75	2.76
RANGE	6.35	156.00	14.15
MAX	12.77	160.00	14.50
MIN	6.42	4.00	.35

\* SUMMARY WITH OUT IERS REMOVED

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	9.80	10.00	1.08
RANGE	6.35	14.00	1.37
MAX	12.77	18.00	1.72
MIN	6.42	4.00	.35

STHP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: FEBRUARY 1, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.5ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FREQ (C/B) X 10E-8
1	21.50	13.58	12.00	.88
2	127.50	21.25	10.00	.47
3	81.60	13.60	14.00	1.03
4	69.50	11.58	13.00	1.12
5	109.20	11.20	10.00	.55
6	105.20	17.70	22.00	1.24
7	77.80	12.97	26.00	2.01
8	93.60	13.93	16.00	1.15
9	90.20	15.13	8.00	.63

NO. OF ANIMALS EQUALS 9  
NO. OF CONTAMINATED EQUALS 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	15.33	14.56	1.00
RANGE	9.67	18.00	1.53
MAX	21.25	26.00	2.01
MIN	11.58	8.00	.47

\* SUMMARY WITH OUTLIERS REMOVED

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	15.62	13.13	.87
RANGE	9.67	11.00	.77
MAX	21.25	22.00	1.24
MIN	11.58	8.00	.67

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA G-46

DOSE LEVEL: POSITIVE CONTROL - DMN - 100 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 1, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.5ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	191.90	31.98	2610.00	81.60
2	72.40	12.07	373.00	30.91
3	82.10	13.68	2015.00	147.26
4	168.40	28.07	2222.00	79.17
5	144.50	24.08	1198.00	49.74
6	96.60	16.10	2323.00	144.26
7	158.50	26.42	1384.00	52.39
8	124.80	20.80	2154.00	103.65
9	141.30	23.55	1390.00	59.02
10	167.50	27.92	2243.00	80.34

NO. OF ANIMALS EQUALS 10

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	22.47	1791.40	82.84
RANGE	19.92	2237.00	116.35
MAX	31.98	2610.00	147.26
MIN	12.07	373.00	30.91

NO OUTLIERS

TOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SALMONELLA  $\beta$ -46

DOSE LEVEL: HIGH ~ 5000 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: FEBRUARY 1, 1974

ANIMAL NUMBER	A RAW CFU X 10E7/0.5ML	B TOTAL CFU X 10E8/1.0ML	C TOTAL NO. MUTANTS X 10E0/1.0ML	D MUTATION FRE (C/B) X 10E-8
1	58.50	9.75	15.00	1.54
2	74.30	12.38	7.00	.57
3	74.10	12.35	11.00	.89
4	40.80	6.80	7.00	1.07
5	85.40	14.23	15.00	1.05
6	99.20	16.53	15.00	.91
7	111.60	18.60	12.00	.67
8	76.90	12.82	16.00	1.25
9	70.50	11.75	11.00	.94

NO. OF ANIMALS EQUALS 9  
TOTAL CFU OUT OF RANGE EQUALS 1

	COL. B (X 10E8)	COL. C (X 10E0)	COL. D (X 10E-8)
MEAN	12.80	12.11	.98
RANGE	11.80	9.00	.97
MAX	18.60	16.00	1.54
MIN	6.80	7.00	.57

NO OUTLIERS

TOP

**HOST MEDIATED ASSAY REPORT SHEET**

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 15, 1974

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	837.00	.64	2.00	2.39
2	542.00	.54	14.00	25.83
3	955.00	.95	14.00	14.66
4	638.00	.64	9.00	14.11
5	688.00	.69	6.00	8.72
6	742.00	.74	18.00	24.26
7	479.00	.48	11.00	22.96
8	655.00	.65	9.00	13.74
9	462.00	.46	6.00	12.99
10	529.00	.53	13.00	24.57
TOTAL		6.53	102.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 15.63

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.65	10.20	16.42
RANGE	.49	18.00	23.44
MAX	.95	18.00	25.83
MIN	.46	2.00	2.39

NO OUTLIERS

S DP

**HOST MEDIATED ASSAY REPORT SHEET**

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: POSITIVE CONTROL -- EMS -- 350 MG/KG I.M.

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 15, 1974

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	628.00	.63	114.00	181.53
2	1576.00	1.58	117.00	74.24
3	686.00	.69	112.00	163.27
4	862.00	.86	93.00	107.89
5	1175.00	1.18	147.00	125.11
6	457.00	.46	40.00	87.53
7	762.00	.76	84.00	110.24
8	1488.00	1.49	207.00	139.11
9	1382.00	1.38	292.00	211.29
10	794.00	.79	93.00	117.13
TOTAL		9.81	1299.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 132.42

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.98	129.90	131.73
RANGE	1.12	252.00	137.05
MAX	1.58	292.00	211.29
MIN	.46	40.00	74.24

NO. OUTLIERS

S\*DP

**HOST MEDIATED ASSAY REPORT SHEET**

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: HIGH - 5000 MG/KG

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: FEBRUARY 15, 1974

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	322.00	.32	6.00	18.63
2	396.00	.40	14.00	35.35
3	846.00	.85	8.00	9.46
4	483.00	.48	9.00	18.63
5	424.00	.42	4.00	9.43
6	642.00	.64	4.00	6.23
7	520.00	.52	7.00	13.46
8	541.00	.54	6.00	11.09
<b>TOTAL</b>		<b>4.17</b>	<b>58.00</b>	

NO. OF ANIMALS EQUALS 8  
 TOTAL SCREENED OUT OF RANGE EQUALS 2

MEAN C/MEAN B = 13.90

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.52	7.25	15.29
RANGE	.52	10.00	29.12
MAX	.85	14.00	35.35
MIN	.32	4.00	6.23

\* SUMMARY WITH OUTLIERS REMOVED

MEAN C/MEAN B = 11.65

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.54	6.29	12.42
RANGE	.52	5.00	12.40
MAX	.85	9.00	18.63
MIN	.32	4.00	6.23

S DP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: NEGATIVE CONTROL - SALINE

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: MAY 3, 1974

ANIMAL NUMBER	RAW CFU X 10 <sup>2</sup> /1.0ML	B TOTAL CFU SCREENED X 10 <sup>2</sup> /1.0ML	C TOTAL RECO BINANTS /1.0ML	D RECOMB. CFU SCREENED X 10 <sup>2</sup> -5
1	484.00	.44	15.00	20.99
2	424.00	.42	8.00	18.87
3	759.00	.76	11.00	14.40
4	896.00	.96	8.00	8.93
5	531.00	.53	8.00	12.58
6	481.00	.48	15.00	31.19
7	437.00	.44	11.00	25.17
8	1745.00	1.75	15.00	8.60
9	652.00	.65	13.00	19.94
10	655.00	.65	11.00	16.79
TOTAL		7.16	115.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 16.05

	COL. B (X 10 <sup>2</sup> )	COL. C (X 10 <sup>0</sup> )	COL. D (X 10 <sup>2</sup> -5)
MEAN	.62	11.50	18.76
RANGE	1.32	7.00	22.59
MAX	1.75	15.00	31.19
MIN	.42	8.00	8.00

NO OUTLIERS

STOP

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: POSITIVE CONTROL -EMS - 350 MG/KG I.M.

TREATMENT: IN VIVO, ORAL, ACUTE

DATE STARTED: MAY 3, 1974

ANIMAL NUMBER	A PAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	1056.00	1.06	73.00	69.13
2	579.00	.58	52.00	89.81
3	646.00	.65	74.00	114.55
4	431.00	.43	44.00	102.09
5	993.00	.99	54.00	54.38
6	655.00	.65	67.00	102.29
7	1050.00	1.05	66.00	62.86
8	945.00	.94	73.00	77.25
9	805.00	.80	93.00	115.53
TOTAL		7.16	596.00	

NO. OF ANIMALS EQUALS 9

NO. OF CONTAMINATED EQUALS 1

MEAN C/MEAN B = 83.24

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.80	66.22	87.54
RANGE	.62	49.00	61.15
MAX	1.06	93.00	115.53
MIN	.43	44.00	54.38

NO OUTLIERS

STOP

SRU'S: 5

SWITCH INS: KS17

JAL

HOST MEDIATED ASSAY REPORT SHEET

COMPOUND: FDA 71-43

ORGANISM: SACCHAROMYCES D-3

DOSE LEVEL: HIGH - 5000 MG/KG

TREATMENT: IN VIVO, ORAL, SUBACUTE

DATE STARTED: MAY 3, 1974

ANIMAL NUMBER	A RAW CFU X 10E5/1.0ML	B TOTAL CFU SCREENED X 10E5/1.0ML	C TOTAL RECOMBINANTS /1.0ML	D RECOMB/CFU SCREENED X 10E-5
1	782.00	.78	16.00	20.46
2	657.00	.66	16.00	24.35
3	210.00	.21	13.00	61.90
4	408.00	.41	14.00	34.31
5	1971.00	1.97	14.00	7.10
6	640.00	.64	20.00	31.25
7	255.00	.26	10.00	39.22
8	693.00	.69	15.00	21.65
9	1538.00	1.54	20.00	13.00
10	363.00	.36	8.00	22.04
TOTAL		7.52	146.00	

NO. OF ANIMALS EQUALS 10

MEAN C/MEAN B = 19.42

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.75	14.60	27.53
RANGE	1.76	12.00	54.80
MAX	1.97	20.00	61.90
MIN	.21	8.00	7.10

\* SUMMARY WITH OUTLIERS REMOVED

MEAN C/MEAN B = 18.20

	COL. B (X 10E5)	COL. C (X 10E0)	COL. D (X 10E-5)
MEAN	.81	14.78	23.71
RANGE	1.72	12.00	32.11
MAX	1.97	20.00	39.22
MIN	.26	8.00	7.10

STOP  
SRU'S: .6

5. Cytogenetics - Test I

a. In vivo

The chromosomal abnormalities observed in the positive controls were significantly higher than either the negative controls or the compound. The maximum effect of the positive control was observed at 48 hours after administering the compound. A depression of the mitotic index was observed in the positive control animals. The effect of the compound on the chromosomes was within normal control values. The frequency of breaks in the negative controls was well within the range that we have seen in the past.

b. In vitro

Anaphase preparations were examined in this test. The positive control compound produced a significantly higher percentage of aberrations on the chromosomes than the negative control or the test compound. Depression of the mitotic index due to the positive compound was not as pronounced as in the in vivo test. The effect of the compound was within normal control values. Negative controls were well within normal limits.

c. CYTOGENETIC SUMMARY SHEETS

CONTRACT FDA 71-268

COMPOUND FDA 71-43

TALC

TEST I



TALC  
 FDA 71-43  
 ACUTE STUDY  
 METAPHASE SUMMARY SHEET  
 TEST I

<u>Compound</u>	<u>Dosage (mg/kg)</u>	<u>Time*</u>	<u>No. of Animals</u>	<u>No. of Cells</u>	<u>Mitotic Index %***</u>	<u>% Cells with Breaks</u>	<u>% Cells with Reunion</u>	<u>% Cells Other Aber.**</u>	<u>% Cells with Aber.**</u>
Negative Control	Saline	6	3	150	15	2	0	0	2
		24	3	150	11	0	0	0	0
		48	3	150	11	0	0	0	0
Usage Level	30	6	5	250	9	0	0	0	0
		24	5	250	12	0	0	0	0
		48	5	250	13	2	0	0	2
Intermediate Level	300	6	5	250	11	0	0	0	0
		24	5	250	8	1	0	0	1
		48	5	250	12	0	0	0	0
LD <sub>5</sub>	3000	6	5	250	8	0	0	0	0
		24	5	250	14	0	0	0	0
		48	5	250	14	0	0	0	0
Positive Control (TEM)	0.3	48	5	250	5	23	14	7(a)	42

\* Time of sacrifice after injection (hours).

\*\* Cells that have polyploidy (P), pulverization (pp), or greater than 10 aberrations (a).

\*\*\* % of cells in mitosis: 500 cells observed/animal.

++ Duplicate aberrations in a single cell will cause this to be a % less than a summation of the % aberration seen.

TALC  
 FDA 71-43  
 SUBACUTE STUDY  
 METAPHASE SUMMARY SHEET  
 TEST I

<u>Compound</u>	<u>Dosage (mg/kg)*</u>	<u>No. of Animals</u>	<u>No. of Cells</u>	<u>Mitotic Index %***</u>	<u>% Cells with Breaks</u>	<u>% Cells with Reunion</u>	<u>% Cells Other Aber. **</u>	<u>% Cells with Aber.</u>
Negative Control	Saline	3	150	9	2	0	0	2
Usage Level	30	5	250	10	0	0	0	0
Intermediate Level	300	5	250	9	1	0	0	1
LD <sub>5</sub>	3000	5	250	12	2	0	0	2

\* Dosage 1X/day X 5 days.

\*\* Cells that have polyploidy (P), pulverization (pp), or greater than 10 aberrations (a).

\*\*\* % of cells in mitosis: 500 cells observed/animal.

TALC  
 FDA 71-43  
 ANAPHASE SUMMARY SHEET  
 TEST I

<u>Compound</u>	<u>Dosage (mcg/ml)</u>	<u>Mitotic Index**</u>	<u>No. of Cells</u>	<u>% Cells with Acentric Frag.</u>	<u>% Cells with Bridges</u>	<u>% Multipolar Cells</u>	<u>% Cells Other Aber.*</u>	<u>% Cells with Aber.**</u>
Low Level	2	4	100	1	0	0	0	1
Medium Level	20	4	100	0	0	0	0	0
High Level	200	3	100	0	0	0	0	0
Negative Control	Saline	3	100	1	0	0	0	1
Positive Control (TEM)	10	2	100	9	9	0	2(pp)	18

\* Cells that have polyploidy (P), pulverization (pp), or greater than 10 aberrations (a).

\*\* % of cells in mitosis: 200 cells observed/dose level.

++ Duplicate aberrations in a single cell will cause this to be a % less than a summation of the % aberration seen.

#### 6. Cytogenetics - Test II

Compound FDA 71-43, Talc, was administered to male rats with an average body weight of 300-350 grams. In the acute study (single dose) and in the subacute study (five doses) a dose of 5000 mg/kg was employed. Meta-phase chromosome spreads were prepared from the bone marrow cells of these animals and scored for chromosomal aberrations. Neither the variety nor the number of these aberrations differed significantly from the negative controls; hence, compound FDA 71-43, Talc, can be considered non-mutagenic as measured by the cytogenetic test.



CYTOGENETIC SUMMARY SHEETS

CONTRACT FDA 71-268

COMPOUND FDA 71-43

TALC

TEST II



TALC  
 FDA 71-43  
 ACUTE STUDY  
 METAPHASE SUMMARY SHEET  
 TEST II

<u>Compound</u>	<u>Dosage (mg/kg)</u>	<u>Time*</u>	<u>No. of Animals</u>	<u>No. of Cells</u>	<u>Mitotic Index %<sup>++</sup></u>	<u>No. of Cells w/ Breaks**</u>	<u>No. of Cells w/ Reunion**</u>	<u>No. of Cells With Other Aberrations**<sup>+</sup></u>	<u>No. of Cells w/ Aber.**</u>
High Level	5000	6 hrs.	5	250	5.06	0	0	0	0
		24 hrs.	5	224	3.17	0	1(0.44)	N.B.	1(0.44)
		48 hrs.	5	250	3.74	1(0.4)	1(0.4)	1pp(0.4),1f(0.4)	3(1.2)
Negative Control	Saline	6 hrs.	3	150	4.26	0	0	1pp(0.6)	1(0.6)
		24 hrs.	3	150	3.53	0	2	4pp(2.6)	6(4.0)
		48 hrs.	3	150	7.20	0	0	0	0
Positive Control (TEM)	0.3	24 hrs.	5	250	4.64	9(3.6)	18(7.2)	3>(1.2) 7f(2.8)	29(11.6)

\* Time of kill after dosing.

\*\* Numbers in ( ) are percent aberrations per total cells counted.

+ Symbols: > = greater than 10 aberrations per cell; f = fragments; pp = polyploid; P = pulverization.

++ Based on a count of at least 500 cells per animal.

N.B. = 49 of the 50 metaphase spreads counted, contained a large metacentric chromosome. This chromosome configuration was not scored as an aberration, but rather was considered "normal" for this particular animal (slide 1991-8).

TALC  
 FDA 71-43  
 SUBACUTE STUDY  
 METAPHASE SUMMARY SHEET  
 TEST II

<u>Compound</u>	<u>Dosage (mg/kg)</u>	<u>No. of Animals</u>	<u>No. of Cells</u>	<u>Mitotic Index %<sup>++</sup></u>	<u>No. of Cells w/ Breaks<sup>**</sup></u>	<u>No. of Cells w/ Reunion<sup>**</sup></u>	<u>No. of Cells w/ Other Aber.<sup>**</sup></u>	<u>No. of Cells w/ Aber.<sup>**</sup></u>
High Level	5000	5	250	2.45	0	1(0.4)	6pp(2.4)	7(2.8)
Negative Control	Saline	3	150	6.15	0	0	0	0

\*\* Numbers in ( ) are percent aberrations per total cells counted.

++ Based on a count of at least 500 cells per animal.

pp = Polyploid.

7. Dominant Lethal Study - Test I

a. Acute study

Significant, dose-related, decreases in average corpora lutea and preimplantation losses were seen in the experimental groups at weeks 4 and 5. Average resorptions showed significant increases in the experimental groups at week 3.

b. Subacute study

Significant increases in average implantations and corpora lutea were seen in the experimental groups at week 4. Average resorptions showed significant, dose-related, increases in the experimental groups at week 6. Significant differences in the proportions of females with one or more and two or more dead implants were also seen at week 6.



c. DOMINANT LETHAL ASSAY SUMMARY TABLES

CONTRACT FDA 71-268

COMPOUND FDA 71-43

TALC

TEST I

(Through error the computer had been programmed so that a double rounding off of numbers occurred at print out. In no way does this alter the statistics which are calculated on the full unrounded numbers.)



TABLE I  
COMPOUND 43 STUDY ACUTE

FERTILITY INDEX

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
!!		1	83/119=0.70	12/20=0.60	7/20=0.35 **	9/20=0.45 *	9/20=0.45 *	10/20=0.50
!!		2	92/119=0.78	11/20=0.55 *	14/20=0.70	14/20=0.70	13/20=0.65	4/20=0.20* **
!!	!	3	96/118=0.82	8/20=0.40 **	14/20=0.70	13/20=0.65	11/20=0.55 **	3/20=0.15 **
!!	!	4	104/120=0.87	14/20=0.70	15/20=0.75	16/19=0.85	16/20=0.80	5/20=0.25* **
		5	95/119=0.80	15/20=0.75	16/20=0.80	18/20=0.90	19/20=0.95	11/20=0.55 *
		6	96/119=0.81	13/20=0.65	18/20=0.90	18/20=0.90	17/20=0.85	16/20=0.80
		7	103/118=0.88	14/20=0.70 *	16/20=0.80	17/20=0.85	15/20=0.75	19/20=0.95*
		8	102/120=0.85	14/20=0.70	14/18=0.78	16/20=0.80	17/20=0.85	18/20=0.90

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !, \* = SIGNIFICANT AT P LESS THAN 0.05

TWO !, \* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE II  
COMPOUND 43 STUDY ACUTE

AVERAGE NUMBER OF IMPLANTATIONS PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
		1	1026/ 83=12.4	154/12=12.8	95/ 7=13.6	119/ 9=13.2	114/ 9=12.7	102/10=10.2* @D
		2	1099/ 92=12.0	124/11=11.3	179/14=12.8* @I	163/14=11.6	156/13=12.0	32/ 4= 8.0
!		3	1178/ 96=12.3	98/ 8=12.3	154/14=11.0 * @D	169/13=13.0	146/11=13.3	37/ 3=12.3
		4	1231/104=11.8	177/14=12.6	178/15=11.9	178/16=11.1	193/16=12.1	54/ 5=10.8
		5	1121/ 95=11.8	169/15=11.3	174/16=10.9	222/18=12.3	239/19=12.6	129/11=11.7
		6	1125/ 96=11.7	167/13=12.9 * @I	220/18=12.2	212/18=11.8	208/17=12.2	193/16=12.1
& !	!	7	1260/103=12.2	176/14=12.6	204/16=12.8	194/17=11.4* @D	173/15=11.5@ D	222/19=11.7
!		8	1192/102=11.7	161/14=11.5	166/14=11.9	212/16=13.3* @I * @I	188/17=11.1	205/18=11.4

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST

! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05

TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE III  
 COMPOUND 43 STUDY ACUTE

AVERAGE CORPORA LUTEA PER PREGNANT FEMALE

LOG ARITH DOSE DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
! ! ! & !	1	1126/ 83=13.6	196/12=16.3 **@@I	105/ 7=15.0	130/ 9=14.4@D	141/ 9=15.7	136/10=13.6*@
! ! !	2	1220/ 92=13.3	139/11=12.6	204/14=14.6*@I	208/14=14.9*@@I @I	191/13=14.7*@I	52/ 4=13.0
! ! ! & ! !	3	1254/ 96=13.1	110/ 8=13.8	185/14=13.2	198/13=15.2 @I	166/11=15.1 *@@I	40/ 3=13.3
! ! !	4	1316/104=12.7	216/14=15.4 **@@I	190/15=12.7**@D	205/16=12.8**@D	204/16=12.8**@D	55/ 5=13.0*@
! ! ! ! ! !	5	1194/ 95=12.6	234/15=15.6 **@@I	201/16=12.6**@D	245/18=13.6**@D	274/19=14.4 *@I	140/11=12.7**
! ! ! ! ! !	6	1233/ 96=12.8	213/13=16.4 **@@I	287/18=15.9 **@@I	295/18=16.4 **@@I	279/17=16.4 **@@I	261/16=16.3 **
! ! ! ! ! !	7	1319/103=12.8	224/14=16.0 **@@I	235/16=14.7 *@@I	239/17=14.1@D *@I	204/15=13.6*@D	309/19=16.3 **
! ! ! ! !	8	1410/102=13.8	189/14=13.5	203/14=14.5	226/16=14.1	205/17=12.1 **@D	227/18=12.6

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST  
 ! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05  
 TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
 &, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE IV  
COMPOUND 43 STUDY ACUTE

AVERAGE PREIMPLANTATION LOSSES PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
1 & !		1	100/ 83= 1.2	42/12= 3.5 *@I	10/ 7= 1.4	11/ 9= 1.2@D	27/ 9= 3.0	34/10= 3.4 *@I
@@!! & !		2	121/ 92= 1.3	15/11= 1.4	25/14= 1.8	45/14= 3.2@I **@@I	35/13= 2.7 @I	20/ 4= 5.0*@@ **@I
@@!! !		3	76/ 96= 0.8	12/ 8= 1.5	31/14= 2.2 **@@I	29/13= 2.2 @I	20/11= 1.8 @I	3/ 3= 1.0
! !		4	85/104= 0.8	39/14= 2.8 **@@I	12/15= 0.8*@@D	27/16= 1.7	11/16= 0.7**@@D	11/ 5= 2.2
@@!! @@!! & !		5	73/ 95= 0.8	65/15= 4.3 **@@I	27/16= 1.7*@@D *@I	23/18= 1.3**@@D	35/19= 1.8*@@D *@I	11/11= 1.0**@
@@!! @@!!		6	108/ 96= 1.1	46/13= 3.5 **@@I	67/18= 3.7 **@@I	83/18= 4.6 **@@I	71/17= 4.2 **@@I	68/16= 4.3 **@
@@!! @@!!		7	59/103= 0.6	48/14= 3.4 **@@I	31/16= 1.9 *@I	45/17= 2.7 **@@I	31/15= 2.1 **@@I	87/19= 4.6 **@
! !		8	218/102= 2.1	28/14= 2.0	37/14= 2.6 @I	14/16= 0.9 @D	17/17= 1.0	22/18= 1.2

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST  
! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05  
TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
! ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE V  
COMPOUND 43 STUDY ACUTE

AVERAGE RESORPTIONS (DEAD IMPLANTS) PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
		1	16/ 83=0.20	4/12=0.34	2/ 7=0.29	2/ 9=0.23	4/ 9=0.45	36/10=3.60**@@I **@@I
		2	35/ 92=0.39	8/11=0.73	7/14=0.50	4/14=0.29	12/13=0.93	3/ 4=0.75
!		3	53/ 96=0.56	0/ 8=0.0 **@@D	6/14=0.43*@@I	5/13=0.39*@@I	5/11=0.46*@@I	6/ 3=2.00@I
!	!	4	46/104=0.45	7/14=0.50	12/15=0.80	7/16=0.44	2/16=0.13 *@@D	13/ 5=2.60
!	!	5	52/ 95=0.55	8/15=0.54	16/16=1.00	6/18=0.34	17/19=0.90 @I	50/11=4.55***@@I ***@@I
		6	40/ 96=0.42	5/13=0.39	6/18=0.34	5/18=0.28	5/17=0.30	20/16=1.25*@I *@@I
		7	45/103=0.44	8/14=0.58	5/16=0.32	6/17=0.36	3/15=0.20	14/19=0.74
		8	56/102=0.55	9/14=0.65	3/14=0.22@D @D	13/16=0.82	8/17=0.48	24/18=1.34 *@@I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST  
! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05  
TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
&, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE VI  
COMPOUND 43 STUDY ACUTE

PROPORTION OF FEMALES WITH ONE OR MORE DEAD IMPLANTATIONS

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
		1	16/ 83=0.20	3/12=0.25	2/ 7=0.29	2/ 9=0.23	3/ 9=0.34	7/10=0.70* **
		2	26/ 92=0.29	6/11=0.55	5/14=0.36	3/14=0.22	4/13=0.31	1/ 4=0.25
		3	32/ 96=0.34	0/ 8=0.0	5/14=0.36	5/13=0.39*	5/11=0.46*	2/ 3=0.67*
		4	34/104=0.33	5/14=0.36	6/15=0.40	4/16=0.25	2/16=0.13	3/ 5=0.60
!	!	5	33/ 95=0.35	3/15=0.20	4/16=0.25	6/18=0.34	12/19=0.64* *	11/11=1.00** **
		6	31/ 96=0.33	5/13=0.39	5/18=0.28	4/18=0.23	4/17=0.24	10/16=0.63 *
		7	33/103=0.33	5/14=0.36	4/16=0.25	6/17=0.36	3/15=0.20	9/19=0.48
		8	37/102=0.37	7/14=0.50	3/14=0.22	6/16=0.38	5/17=0.30	13/18=0.73 **

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE VII  
 COMPOUND 43 STUDY ACUTE

PROPORTION OF FEMALES WITH TWO OR MORE DEAD IMPLANTATIONS

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
!	!!	1	0/ 83=0.0	1/12=0.09 **	0/ 7=0.0	0/ 9=0.0	1/ 9=0.12 **	7/10=0.70** **
!	!!	2	9/ 92=0.10	2/11=0.19	2/14=0.15	1/14=0.08	1/13=0.08	1/ 4=0.25
!	!	3	16/ 96=0.17	0/ 8=0.0	1/14=0.08	0/13=0.0	0/11=0.0	2/ 3=0.67* *
!	!	4	9/104=0.09	2/14=0.15	3/15=0.20	2/16=0.13	0/16=0.0	3/ 5=0.60* **
!	!	5	14/ 95=0.15	2/15=0.14	3/16=0.19	0/18=0.0	3/19=0.16	9/11=0.82** **
!	!	6	9/ 96=0.10	0/13=0.0	1/18=0.06	1/18=0.06	1/17=0.06	6/16=0.38* **
!	!	7	8/103=0.08	3/14=0.22	1/16=0.07	0/17=0.0 *	0/15=0.0	3/19=0.16
!	!	8	16/102=0.16	2/14=0.15	0/14=0.0	4/16=0.25	2/17=0.12	3/18=0.17

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE VIII  
COMPOUND 43 STUDY ACUTE

DEAD IMPLANTS / TOTAL IMPLANTS

WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG	POSITIVE CONTROL
1	16/1026=0.02	4/154=0.03	2/95=0.03	2/119=0.02	4/114=0.04	36/102=0.36*
2	35/1099=0.04	8/124=0.07	7/179=0.04	4/163=0.03	12/156=0.08	3/32=0.10
3	53/1178=0.05	0/98=0.0 **@D	6/154=0.04*@D	5/169=0.03*@D	5/146=0.04@D	6/37=0.17
4	46/1231=0.04	7/177=0.04	12/178=0.07	7/178=0.04	2/193=0.02 **@D	13/54=0.25
5	52/1121=0.05	8/169=0.05	16/174=0.10	6/222=0.03 @D	17/239=0.08	50/129=0.39*
6	40/1125=0.04	5/167=0.03	6/220=0.03	5/212=0.03	5/208=0.03	20/193=0.11* @D
7	45/1260=0.04	8/176=0.05	5/204=0.03	6/194=0.04	3/173=0.02 @D	14/222=0.07
8	56/1192=0.05	9/161=0.06	3/166=0.02@D *@D	13/212=0.07	8/188=0.05	24/205=0.12*

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT DIFFERENCES USING THE HISTORICAL CONTROL GROUP

\* = TWO-TAILED TEST  
@ = ONE-TAILED TEST

ONE \*,@ = SIGNIFICANT AT P LESS THAN 0.05  
TWO \*,@ = SIGNIFICANT AT P LESS THAN 0.01

\*,@ SIGNIFICANTLY DIFFERENT FROM CONTROL

TABLE I  
 COMPOUND 43 STUDY SUBACUTE

FERTILITY INDEX

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
!!		1	82/119=0.69	10/20=0.50	8/20=0.40*	10/20=0.50	5/20=0.25**
!!		2	89/120=0.75	15/20=0.75	15/20=0.75	15/20=0.75	15/20=0.75
		3	89/119=0.75	12/20=0.60	13/20=0.65	12/20=0.60	13/20=0.65
		4	91/114=0.80	13/20=0.65	15/20=0.75	16/20=0.80	16/20=0.80
		5	92/119=0.78	16/20=0.80	15/20=0.75	19/20=0.95	15/20=0.75
		6	101/119=0.85	19/20=0.95	19/20=0.95	19/20=0.95	19/20=0.95
		7	100/115=0.87	17/20=0.85	19/20=0.95	19/20=0.95	17/20=0.85

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE II  
COMPOUND 43 STUDY SUBACUTE

AVERAGE NUMBER OF IMPLANTATIONS PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
		1	966/ 82=11.8	118/10=11.8	104/ 8=13.0 * $\partial$ I	125/10=12.5	65/ 5=13.0
		2	1115/ 89=12.5	186/15=12.4	194/15=12.9	197/15=13.1	185/15=12.3
		3	1049/ 89=11.8	147/12=12.3	153/13=11.8	137/12=11.4	163/13=12.5
!		4	1085/ 91=11.9	136/13=10.5 * $\partial$ D	179/15=11.9 $\partial$ I	194/16=12.1 $\partial$ I	192/16=12.0 $\partial$ I
		5	1110/ 92=12.1	189/16=11.8	185/15=12.3	233/19=12.3	183/15=12.2
		6	1191/101=11.8	246/19=13.0	215/19=11.3 $\partial$ D	231/19=12.2	241/19=12.7
& !!	!	7	1138/100=11.4	214/17=12.6 $\partial$ I	222/19=11.7	234/19=12.3 * $\partial$ I	213/17=12.5 * $\partial\partial$ I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST

! AND  $\partial$  = ONE-TAILED TEST

ONE !, &,  $\partial$ , \* = SIGNIFICANT AT P LESS THAN 0.05

TWO !, &,  $\partial$ , \* = SIGNIFICANT AT P LESS THAN 0.01

\* $\partial$  SIGNIFICANTLY DIFFERENT FROM CONTROL

&, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE III  
 COMPOUND 43 STUDY SUBACUTE

AVERAGE CORPORA LUTEA PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
		1	1079/ 82=13.2	139/10=13.9	112/ 8=14.0	137/10=13.7	72/ 5=14.4
! & !		2	1189/ 89=13.4	206/15=13.7	219/15=14.6 *@I	223/15=14.9 *@@I	211/15=14.1
! & !		3	1125/ 89=12.6	165/12=13.8	180/13=13.9	164/12=13.7	179/13=13.8 @I
@@!! @@!! & !		4	1134/ 91=12.5	151/13=11.6 @D	189/15=12.6	232/16=14.5**@@I **@@I	220/16=13.8**@@I *@I
! & ! @@!! & @!!		5	1157/ 92=12.6	209/16=13.1	206/15=13.7 *@I	243/19=12.8	219/15=14.6@I **@@I
@@!! & @!!		6	1268/101=12.6	312/19=16.4 **@@I	298/19=15.7 **@@I	274/19=14.4 *@@I	323/19=17.0 **@@I
! @@!!		7	1215/100=12.2	259/17=15.2 **@@I	281/19=14.8 **@@I	268/19=14.1 *@@I	230/17=13.5@D **@@I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

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TWO !, @, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL

@, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE IV  
COMPOUND 43 STUDY SUBACUTE

AVERAGE PREIMPLANTATION LOSSES PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
		1	113/ 82= 1.4	21/10= 2.1	8/ 8= 1.0	12/10= 1.2	7/ 5= 1.4
εε!!	!	2	74/ 89= 0.8	20/15= 1.3	25/15= 1.7 *@I	26/15= 1.7 *@I	26/15= 1.7 @I
εε!!		3	76/ 89= 0.9	18/12= 1.5 *@I	27/13= 2.1 **@@I	27/12= 2.3 **@@I	16/13= 1.2
εε!!	εε!!	4	49/ 91= 0.5	15/13= 1.2	10/15= 0.7	38/16= 2.4@I **@@I	29/16= 1.8 *@I
εε!!	εε!!	5	47/ 92= 0.5	20/16= 1.3 *@@I	21/15= 1.4 **@@I	10/19= 0.5@D	36/15= 2.4 **@@I
εε!!	εε!!	6	77/101= 0.8	66/19= 3.5 **@@I	83/19= 4.4 **@@I	43/19= 2.3 **@@I	82/19= 4.3 **@@I
!	ε !	7	77/100= 0.8	45/17= 2.7 *@I	59/19= 3.1 **@@I	34/19= 1.8 @I	17/17= 1.0

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ε AND \* = TWO-TAILED TEST

! AND @ = ONE-TAILED TEST

ONE !, ε, @, \* = SIGNIFICANT AT P LESS THAN 0.05

TWO !, ε, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL

ε, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE V  
 COMPOUND 43 STUDY SUBACUTE

AVERAGE RESORPTIONS (DEAD IMPLANTS) PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
		1	33/ 82=0.41	2/10=0.20	2/ 8=0.25	9/10=0.90	2/ 5=0.40
		2	45/ 89=0.51	4/15=0.27	8/15=0.54	7/15=0.47	9/15=0.60 <sup>Ⓢ</sup>
		3	47/ 89=0.53	8/12=0.67	6/13=0.47	5/12=0.42	9/13=0.70
		4	51/ 91=0.57	10/13=0.77	7/15=0.47	17/16=1.07 * <sup>Ⓢ</sup>	11/16=0.69
		5	56/ 92=0.61	15/16=0.94	8/15=0.54	14/19=0.74	12/15=0.80
ⓈⓈⓈ Ⓢ !!	!	6	46/101=0.46	1/19=0.06 ** <sup>Ⓢ</sup> Ⓢ	12/19=0.64** <sup>Ⓢ</sup> Ⓢ	15/19=0.79** <sup>Ⓢ</sup> Ⓢ	15/19=0.79** <sup>Ⓢ</sup> Ⓢ
		7	52/100=0.52	7/17=0.42	10/19=0.53	19/19=1.00	11/17=0.65

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

Ⓢ AND \* = TWO-TAILED TEST  
 ! AND Ⓢ = ONE-TAILED TEST

ONE !, Ⓢ, Ⓢ, \* = SIGNIFICANT AT P LESS THAN 0.05  
 TWO !, Ⓢ, Ⓢ, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, Ⓢ SIGNIFICANTLY DIFFERENT FROM CONTROL  
 Ⓢ, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE VI  
COMPOUND 43 STUDY SUBACUTE

PROPORTION OF FEMALES WITH ONE OR MORE DEAD IMPLANTATIONS

LOG ARITH DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
		1	27/ 82=0.33	1/10=0.10	2/ 8=0.25	5/10=0.50	1/ 5=0.20
		2	29/ 89=0.33	3/15=0.20	5/15=0.34	4/15=0.27	8/15=0.54
		3	30/ 89=0.34	4/12=0.34	4/13=0.31	4/12=0.34	7/13=0.54
		4	30/ 91=0.33	8/13=0.62	5/15=0.34	10/16=0.63	7/16=0.44
		5	39/ 92=0.43	10/16=0.63	4/15=0.27*	8/19=0.43	6/15=0.40
!	!	6	32/101=0.32	1/19=0.06	9/19=0.48**	10/19=0.53**	10/19=0.53**
!	!	7	28/100=0.28	6/17=0.36	9/19=0.48	8/19=0.43	9/17=0.53

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (READING OF COLUMN)

TABLE VII  
COMPOUND 43 STUDY SUBACUTE

PROPORTION OF FEMALES WITH TWO OR MORE DEAD IMPLANTATIONS

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
		1	5/ 82=0.07	1/10=0.10	0/ 8=0.0	1/10=0.10	1/ 5=0.20
		2	7/ 89=0.08	1/15=0.07	1/15=0.07	3/15=0.20	1/15=0.07
		3	10/ 89=0.12	4/12=0.34 *	2/13=0.16	1/12=0.09	2/13=0.16
		4	12/ 91=0.14	2/13=0.16	2/15=0.14	5/16=0.32	3/16=0.19
		5	14/ 92=0.16	4/16=0.25	3/15=0.20	4/19=0.22	3/15=0.20
!	!	6	9/101=0.09	0/19=0.0	1/19=0.06	5/19=0.27*	5/19=0.27*
		7	13/100=0.13	1/17=0.06	1/19=0.06	5/19=0.27	1/17=0.06

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE VIII  
 COMPOUND 43 STUDY SUBACUTE

DEAD IMPLANTS / TOTAL IMPLANTS

WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 30.000 MG/KG	DOSE LEVEL 300.000 MG/KG	DOSE LEVEL 3000.000 MG/KG
1	33/966=0.04	2/118=0.02	2/104=0.02	9/125=0.08	2/65=0.04
2	45/1115=0.05	4/186=0.03	8/194=0.05	7/197=0.04	9/185=0.05
3	47/1049=0.05	8/147=0.06	6/153=0.04	5/137=0.04	9/163=0.06
4	51/1085=0.05	10/136=0.08	7/179=0.04	17/194=0.09	11/192=0.06
5	56/1110=0.06	15/189=0.08	8/185=0.05	14/233=0.07	12/183=0.07
6	46/1191=0.04	1/246=0.01	12/215=0.06* $\alpha$ I	15/231=0.07** $\alpha\beta$ I	15/241=0.07** $\alpha\beta$ I
		** $\alpha\beta$ D			
7	52/1138=0.05	7/214=0.04	10/222=0.05	19/234=0.09	11/213=0.06

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT DIFFERENCES USING THE HISTORICAL CONTROL GROUP

\* = TWO-TAILED TEST  
 $\alpha$  = ONE-TAILED TEST

ONE \*, $\alpha$  = SIGNIFICANT AT P LESS THAN 0.05  
 TWO \*, $\beta$  = SIGNIFICANT AT P LESS THAN 0.01

\*, $\alpha$  SIGNIFICANTLY DIFFERENT FROM CONTROL

#### 8. Dominant Lethal Study - Test II

Compound FDA 71-43, Talc, was administered to ten male rats (400 grams) at a dose level of 5,000 mg/kg according to acute (single dose) and subacute (five doses) protocols. Each treated male rat was mated with two virgin female rats each week for seven (subacute) or eight (acute) weeks. Two weeks after mating, female rats were sacrificed and the fertility index, preimplantation loss and lethal effects on the embryos were determined and compared with those same parameters calculated from negative (saline-dosed) and positive (0.3 mg/kg TEM-dosed) control animals.

The values calculated for those parameters from animals dosed with compound FDA 71-43, Talc, did not significantly vary from those obtained from the negative controls; whereas, TEM caused a significant pre-implantation loss and embryo resorption during the first five weeks.

Comparing these data with the previously obtained values for dose levels of 3,000 mg/kg, 300 mg/kg and 30 mg/kg revealed no dose-response or time-trend patterns, thus indicating that compound FDA 71-43, Talc, does not induce dominant lethal mutations as measured by this test.



DOMINANT LETHAL ASSAY SUMMARY TABLES

CONTRACT FDA 71-268

COMPOUND FDA 71-43

TALC

TEST II

(Through error the computer had been programmed so that a double rounding off of numbers occurred at print out. In no way does this alter the statistics which are calculated on the full unrounded numbers.)





TABLE II  
 COMPOUND 43 STUDY ACUTE

AVERAGE NUMBER OF IMPLANTATIONS PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG	POSITIVE CONTROL
		1	1351/109=12.4	189/ 15=12.6	190/ 14=13.6 @I	102/ 12= 8.5**@@D **@@D
		2	1427/119=12.0	202/ 17=11.9	215/ 16=13.4@I **@@I	144/ 15= 9.6@D *@@D
		3	1435/119=12.1	196/ 16=12.3	175/ 14=12.5	97/ 19= 5.1**@@D **@@D
		4	1626/136=12.0	219/ 18=12.2	156/ 13=12.0	65/ 11= 5.9**@@D **@@D
		5	1466/127=11.5	241/ 18=13.4 **@@I	213/ 17=12.5	195/ 15=13.0 *@I
		6	1512/128=11.8	202/ 15=13.5 **@@I	192/ 15=12.8 @I	250/ 19=13.2 **@@I
		7	1626/133=12.2	219/ 17=12.9	190/ 16=11.9	176/ 14=12.6
		8	1551/133=11.7	234/ 17=13.8 **@@I	206/ 16=12.9 *@@I	236/ 18=13.1 **@@I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST  
 ! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05  
 TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
 &, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE III  
 COMPOUND 43 STUDY ACUTE

AVERAGE CORPORA LUTEA PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG	POSITIVE CONTROL
		1	1504/109=13.8	228/ 15=15.2 *@I	214/ 14=15.3 *@@I	176/ 12=14.7
		2	1588/119=13.3	250/ 17=14.7 *@@I	243/ 16=15.2 **@@I	215/ 15=14.3
		3	1565/119=13.2	234/ 16=14.6 *@I	185/ 14=13.2	252/ 19=13.3
		4	1784/136=13.1	272/ 18=15.1 **@@I	184/ 13=14.2	166/ 11=15.1 *@I
		5	1648/127=13.0	266/ 18=14.8 *@@I	258/ 17=15.2 **@@I	220/ 15=14.7 *@@I
		6	1689/128=13.2	235/ 15=15.7 **@@I	219/ 15=14.6 *@I	325/ 19=17.1 **@@I
		7	1767/133=13.3	246/ 17=14.5 **@@I	231/ 16=14.4 @I	197/ 14=14.1
		8	1823/133=13.7	266/ 17=15.7 **@@I	257/ 16=16.1 *@I	278/ 18=15.4 **@@I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

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!\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL

&, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE IV  
COMPOUND 43 STUDY ACUTE

AVERAGE PREIMPLANTATION LOSSES PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG	POSITIVE CONTROL
		1	153/109= 1.4	39/ 15= 2.6 @I	24/ 14= 1.7	74/ 12= 6.2@I **@@I
		2	161/119= 1.4	48/ 17= 2.8	28/ 16= 1.8 @I	71/ 15= 4.7*@@I **@@I
		3	130/119= 1.1	38/ 16= 2.4	10/ 14= 0.7@D	155/ 19= 8.2***@@I **@@I
		4	158/136= 1.2	53/ 18= 2.9 **@@I	28/ 13= 2.2	101/ 11= 9.2***@@I **@@I
		5	182/127= 1.4	25/ 18= 1.4	45/ 17= 2.7 *@I	25/ 15= 1.7 @I
		6	177/128= 1.4	33/ 15= 2.2 *@I	27/ 15= 1.8	75/ 19= 4.0 **@@I
		7	141/133= 1.1	27/ 17= 1.6	41/ 16= 2.6 *@I	21/ 14= 1.5
		8	272/133= 2.1	32/ 17= 1.9	51/ 16= 3.2	42/ 18= 2.3 @I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

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TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
&, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)



COMPOUND 43                      TABLE VI  
STUDY ACUTE

PROPORTION OF FEMALES WITH ONE OR MORE DEAD IMPLANTATIONS

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG	POSITIVE CONTROL
		1	24/109=0.23	2/ 15=0.14	5/ 14=0.36	12/ 12=1.00** **
		2	38/119=0.32	8/ 17=0.48	9/ 16=0.57	15/ 15=1.00** **
		3	39/119=0.33	5/ 16=0.32	5/ 14=0.36	19/ 19=1.00** **
		4	46/136=0.34	12/ 18=0.67 **	4/ 13=0.31*	11/ 11=1.00* **
		5	45/127=0.36	6/ 18=0.34	6/ 17=0.36	15/ 15=1.00** **
		6	44/128=0.35	8/ 15=0.54	8/ 15=0.54	15/ 19=0.79 **
		7	46/133=0.35	8/ 17=0.48	5/ 16=0.32	10/ 14=0.72 **
		8	50/133=0.38	4/ 17=0.24	6/ 16=0.38	11/ 18=0.62*

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !, \* = SIGNIFICANT AT P LESS THAN 0.05

TWO !, \* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE VII  
COMPOUND 43                      STUDY ACUTE

PROPORTION OF FEMALES WITH TWO OR MORE DEAD IMPLANTATIONS

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG	POSITIVE CONTROL
		1	3/109=0.03	0/ 15=0.0	2/ 14=0.15 *	12/ 12=1.00** **
		2	14/119=0.12	3/ 17=0.18	5/ 16=0.32 *	15/ 15=1.00** **
		3	17/119=0.15	4/ 16=0.25	1/ 14=0.08	15/ 19=0.79** **
		4	12/136=0.09	1/ 18=0.06	1/ 13=0.08	11/ 11=1.00** **
		5	18/127=0.15	3/ 18=0.17	2/ 17=0.12	13/ 15=0.87** **
		6	13/128=0.11	4/ 15=0.27	1/ 15=0.07	10/ 19=0.53 **
		7	14/133=0.11	3/ 17=0.18	1/ 16=0.07	2/ 14=0.15
		8	18/133=0.14	1/ 17=0.06	3/ 16=0.19	6/ 18=0.34* *

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)





TABLE II  
 COMPOUND 43                      STUDY SUBACUTE

AVERAGE NUMBER OF IMPLANTATIONS PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG
	WEEK			
	1	1231/104=11.8	197/ 14=14.1 **@I	190/ 15=12.7*@@D @I
	2	1474/118=12.5	189/ 16=11.8	230/ 18=12.8
	3	1405/119=11.8	218/ 17=12.8	190/ 15=12.7
	4	1414/120=11.8	176/ 15=11.7	208/ 17=12.2
	5	1462/122=12.0	209/ 17=12.3	231/ 18=12.8 @I
	6	1626/136=12.0	170/ 14=12.1	213/ 18=11.8
	7	1566/135=11.6	170/ 16=10.6	238/ 18=13.2*@I **@I

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST

! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05

TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL

!, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE III  
COMPOUND 43                      STUDY SUBACUTE

AVERAGE CORPORA LUTEA PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG
1	1385/104=13.3	212/ 14=15.1	225/ 15=15.0	
			**@I	*@I
2	1599/118=13.6	239/ 16=14.9	254/ 18=14.1	
			*@I	
3	1535/119=12.9	246/ 17=14.5	238/ 15=15.9	
			**@I	**@I
4	1499/120=12.5	208/ 15=13.9	238/ 17=14.0	
		@I	*@I	
5	1554/122=12.7	286/ 17=16.8	288/ 18=16.0	
		**@I	**@I	
6	1809/136=13.3	220/ 14=15.7	251/ 18=13.9	@D
		**@I		
7	1711/135=12.7	244/ 16=15.3	260/ 18=14.4	
		**@I	**@I	

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

& AND \* = TWO-TAILED TEST  
! AND @ = ONE-TAILED TEST

ONE !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.05  
TWO !, &, @, \* = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
&, ! SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)

TABLE IV  
COMPOUND 43                      STUDY SUBACUTE

AVERAGE PREIMPLANTATION LOSSES PER PREGNANT FEMALE

LOG DOSE	ARITH DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG
		1	154/104= 1.5	15/ 14= 1.1	35/ 15= 2.3
		2	125/118= 1.1	50/ 16= 3.1 **@!I	24/ 18= 1.3*!@D
		3	130/119= 1.1	28/ 17= 1.7	48/ 15= 3.2 *!@!I
		4	85/120= 0.7	32/ 15= 2.1 *!I	30/ 17= 1.8 *!@!I
		5	92/122= 0.8	77/ 17= 4.5 **@!I	57/ 18= 3.2 **@!I
		6	183/136= 1.4	50/ 14= 3.6 **@!I	38/ 18= 2.1!@D
		7	145/135= 1.1	74/ 16= 4.6 **@!I	22/ 18= 1.2**@!@D

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

! AND \* = TWO-TAILED TEST  
! AND @ = ONE-TAILED TEST

ONE !, @, \*, = SIGNIFICANT AT P LESS THAN 0.05  
TWO !, @, \*, = SIGNIFICANT AT P LESS THAN 0.01

\*, @ SIGNIFICANTLY DIFFERENT FROM CONTROL  
!, !: SIGNIFICANT RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)



TABLE VI  
 COMPOUND 43                      STUDY SUBACUTE

PROPORTION OF FEMALES WITH ONE OR MORE DEAD IMPLANTATIONS

LOG ARITH DOSE DOSE	WEEK	HISTORICAL CONTROL	NEGATIVE CONTROL	DOSE LEVEL 5000. MG/KG
1	31/104=0.30	6/ 14=0.43	4/ 15=0.27	
2	38/118=0.33	7/ 16=0.44	9/ 18=0.50	
3	42/119=0.36	9/ 17=0.53	5/ 15=0.34	
4	42/120=0.35	9/ 15=0.60	7/ 17=0.42	
5	54/122=0.45	5/ 17=0.30	5/ 18=0.28	
6	43/136=0.32	8/ 14=0.58	11/ 18=0.62	*
7	42/135=0.32	8/ 16=0.50	9/ 18=0.50	

SYMBOLS ON FIRST LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE NEGATIVE CONTROL GROUP

SYMBOLS ON SECOND LINE DENOTE SIGNIFICANT RELATIONSHIPS AND DIFFERENCES USING THE HISTORICAL CONTROL GROUP

ONE !,\* = SIGNIFICANT AT P LESS THAN 0.05

TWO !,\* = SIGNIFICANT AT P LESS THAN 0.01

\* SIGNIFICANTLY DIFFERENT FROM CONTROL

! SIGNIFICANT LINEAR RELATIONSHIP WITH ARITH OR LOG DOSE (HEADING OF COLUMN)





## APPENDICES

### II. MATERIALS AND METHODS

#### A. Animal Husbandry

##### 1. Animals (Rats and Mice)

Ten to twelve week old rats (280 to 350 g) and male mice (25 to 30 g) were fed a commercial 4% fat diet and water ad libitum until they were put on experiment. Flow Laboratories random-bred, closed colony, Sprague-Dawley CD strain rats were used in the cytogenetic studies. Flow Laboratories ICR male mice were employed in the Host-Mediated Assay.

##### 2. Preparation of Diet

A commercial 4% fat diet was fed to all animals. Periodic tests to verify the absence of coliforms, Salmonella and Pseudomonas sp. were performed.

##### 3. Husbandry

Animals were held in quarantine for 4-11 days. Mice were housed five to a cage and rats one to five to a cage. Animals were identified by ear punch. Sanitary cages and bedding were used, and changed two times per week, at which time water containers were cleaned, sanitized and filled. Once a week, cages were repositioned on racks; racks were repositioned within rooms monthly. Personnel handling animals or working within animal facilities wore head coverings and face masks, as well as suitable garments. Individuals with respiratory or other overt infections were excluded from the animal facilities.

#### B. Dosage Determination

##### 1. Acute LD<sub>50</sub> and LD<sub>5</sub> Determination

Since the compounds proposed for testing are included in



the food additive regulations as "generally recognized as safe" (GRAS), it was expected that a large number of them would be sufficiently non-toxic so that determination of a  $LD_{50}$  or a  $LD_5$  would be of no practical value. In fact, this has been our experience with previously tested compounds from this list. In the case of these relatively non-toxic compounds, attempts were made to assure that the amounts to be administered would not affect the animals by means (mechanical, physical, etc.) related to their bulk rather than to their toxicity. In the cases of certain compounds where a  $LD_{50}$  or a  $LD_5$  could not be determined, an exceedingly high concentration, 5 g/kg, was employed and accepted as the  $LD_5$  level. In cases where the toxicity was high enough to allow determination of a  $LD_5$ , the following protocol was used.

Thirty rats of the strain chosen for studies described below and of approximately the age and weight specified were assigned at random to six groups. Each group was then given, using the chosen route of administration, one of a series of dosages of the test compound following a logarithmic dosage scheme. The series of dosages were derived from a consideration of whatever toxicity information was available for the particular test compound. The objective in selecting dosages was to choose values which would cause mortalities between 10% and 90%.

When information was inadequate to derive a suitable series of dosages, five rats were used to identify the proper range. Each of these was given one of a widely spaced (differing by 10X) series of doses. This was confidently expected to suffice for derivation of the series of dosages to be used in the  $LD_{50}$  determination.

The mortalities observed when the series of dosages were given to the 30 rats were then subjected to a probit analysis and calculation of  $LD_{50}$ ,  $LD_5$ , slope and confidence limits by the method of Litchfield and Wilcoxon. The highest dose level used was either a finite  $LD_5$  or 5000 mg/kg. The intermediate level used was either 1/10 of the finite  $LD_5$  or 2500 mg/kg. The low level used was either 1/100 of the finite  $LD_5$  or 30 mg/kg.

## 2. Subacute Studies

Subacute doses were identical to those used in the acute studies. Each subacute study animal was given the acute dosage once a day for each of five consecutive days (24 hours apart).

## C. Mutagenicity Testing Protocols

### 1. Host-Mediated Assay

Flow Laboratories ICR random-bred male mice were used in this study. In the acute and subacute studies ten animals, 25-30 g each, were employed at each dose level. Solvent and positive controls were run at all times. The positive control (dimethyl nitrosamine) was run by the acute system only at a dose of 100 mg/kg for Salmonella. For yeast, ethyl methane sulfonate (EMS) intramuscularly injected at a dose of 350 mg/kg was used. The solvents used and the toxicity data are presented in the Results and Discussion Section of the report.

The indicator organisms used in this study were: (1) two histidine auxotrophs (his G-46, TA-1530) of Salmonella typhimurium, and (2) a diploid strain (D-3) of Saccharomyces cerevisiae. The induction of reverse mutation was determined with the Salmonella; mitotic recombination was determined with yeast. Chemicals were evaluated directly by in vitro bacterial and yeast studies prior to, or concurrent with, the studies in



mice. Only animals on the subacute studies were not fed the evening prior to compound administration. The Salmonella were carried in tryptone yeast extract gel, transferred weekly. They were transferred to tryptone yeast extract broth 48 hours before use: they were transferred a second time from broth to broth 24 hours prior to use, and again 8 hours before use. The mouse inoculum was prepared by transferring 4 ml of the 8-hour broth culture to 50 ml broth bottles which had been prewarmed at 37°C. Exponential log-phase organisms were inoculated intraperitoneally into the mice approximately 2-1/2 hours later when the appropriate density indicating  $3.0 \times 10^8$  cells/ml was reached. The Saccharomyces was carried in yeast complete agar. The inoculum was prepared by harvesting the organisms from the surface of the plates with sterile saline. The cells were washed three times with sterile saline and suspended in a concentration of  $5.0 \times 10^8$  cells/ml. Two ml of the suspension was inoculated into each mouse intraperitoneally. Total plate counts on Salmonella were on tryptone yeast extract and for Saccharomyces on yeast complete medium.

a. Acute study

Three dosage levels (usage, intermediate [determined as discussed previously], and  $LD_5$ ) were administered orally by intubation to ten mice. Positive controls and negative vehicle controls were included in each study. All animals received 2 ml of the indicator organism intraperitoneally. Each ml contained  $3.0 \times 10^8$  cells for Salmonella and  $5.0 \times 10^8$  cells for Saccharomyces. Three hours later, each animal was killed and 2 ml of sterile saline was introduced intraperitoneally. As much fluid as possible was then aseptically removed from the peritoneal cavity. Dilution blanks for bacteria containing 4.5 ml of sterile saline were prepared in advance. Tenfold serial

dilutions were made of each peritoneal exudate (0.5 ml exudate + 4.5 ml saline) yielding a concentration series from  $10^0$  (undiluted peritoneal exudate) through  $10^{-7}$ . For enumeration of total bacterial counts, the  $10^{-6}$  and  $10^{-7}$  dilutions were plated on tryptone yeast extract agar, 3 plates/sample, 0.2 ml sample/plate. Each sample was spread over the surface of the plate using a bent glass rod immersed in 95% ethanol and flamed just prior to use. In plating for the total mutant counts on minimal agar, the  $10^0$  dilution was used, 0.2 ml being plated on each of 5 plates. The plating procedure was identical to that followed for the tryptone yeast extract agar plates. All plates were incubated at 37°C, tryptone yeast extract agar plates for 18 hours and minimal agar plates for 40 hours. For yeast mitotic recombination, dilution blanks containing 4.5 ml of sterile saline were prepared in advance. Tenfold serial dilutions were made of each sample yielding a series from  $10^0$  to  $10^{-5}$ . Samples of 0.1 ml of the  $10^{-5}$ ,  $10^{-4}$ , and  $10^{-3}$  dilutions were removed and plated on complete medium (10 plates each). All plates were incubated at 30°C for 40 hours. The  $10^{-5}$  dilutions were used to determine total populations and the  $10^{-4}$  and  $10^{-3}$  plates were examined after an additional 40 hours at 4°C for red sectors indicating a mutation. Bacterial scoring was calculated as follows:

Total mutants on 5 plates x appropriate exponent =  
 CFU/ml (CFU is Colony Forming Units) of sample plated  
 CFU/ml x one/dilution factor ( $10^0 - 10^{-7}$ ) = CFU/ml in undiluted exudate. The mutation frequency (MF) calculated for each sample was:

$$MF = \frac{\text{total mutant cells}}{\text{total population}}$$

$$MF_t/MF_c = \frac{MF \text{ of experimental sample}}{MF \text{ of control sample}}$$

( $MF_t/MF_c = 1.00$  for control sample)



Yeast mitotic recombinants (presumptive ade 2, his 8 homozygotes) were seen as red colonies or as red sectors on a normally white yeast colony. The plates (from  $10^{-4}$  and  $10^{-3}$  dilutions) were scanned under the 10X lens of a dissecting scope to enumerate the red colonies and sectors. Population determinations were made from the  $10^{-5}$  dilution plates. A recombinant frequency (RF) was calculated:

$$RF = \frac{\text{total recombinants counted}}{\text{total number colonies screened}}$$

b. Subacute study

Similar groups of animals at each dose level received five oral doses of the test compound 24 hours apart. Within 30 minutes after the last dosing, the animals were inoculated with the test organism and handled in the same fashion as those in the acute study.

c. In vitro study

Cultures of S. typhimurium histidine auxotrophs (G-46 and TA-1530) were plated on appropriate media. The test compound was then added to the plate, either in the form of a microdrop of solution (0.01 to 0.25 ml) applied to a small filter paper disc resting on the agar or a small crystal applied directly to the agar. Tenfold serial dilutions of the culture were employed and plated so as not to miss the optimum cell density for mutant growth. Mutant colonies were observed and scored. Strain D-3 Saccharomyces cells at proper dilutions were shaken with the test compound, diluted, and plated at 50% survival level or above (see HMA Supplementary Materials and Methods). Red sectors were then scored and the frequency calculated after suitable incubation. Negative and positive controls were run concurrently. The positive control was EMS for Salmonella and Saccharomyces. The in vitro Salmonella tests were reported

as (+) or (-) or questionable; the in vitro Saccharomyces tests were reported as sample concentrations, percent survival, and recombinants/10<sup>5</sup> survivors. For the Saccharomyces a 50% survival level, e.g., an arbitrary 5.0% w/y test level, was used when no LD<sub>50</sub> was determinable.

2. Cytogenetic Studies

a. In vivo study

Ten to twelve week old, male, albino rats obtained from a closed colony (random-bred) were used. A total of 59 animals in the acute study and 18 animals in the subacute study was used, as illustrated in the following protocol.

Number of Animals Used

Acute Study

<u>Treatment</u>	<u>Time Killed After Administration</u>		
	<u>6 Hours</u>	<u>24 Hours</u>	<u>48 Hours</u>
High Level	5	5	5
Intermediate Level	5	5	5
Low Level	5	5	5
Positive Control	0	0	5
Negative Control	3	3	3

Subacute Study

Five doses 24 hours apart; animals killed 6 hours after last dose.

<u>Treatment</u>	<u>Killed After Administration</u>
High Level	5
Intermediate Level	5
Low Level	5
Negative Control	3

All animals were dosed by gastric intubation.

Four hours after the last compound administration, and two hours prior to killing, each animal was given 4 mg/kg of colcemid intra-

peritoneally in order to arrest the bone marrow cells in C-mitosis. Animals were killed by using CO<sub>2</sub>, and the adhering muscle and epiphysis of one femur were removed. The marrow "plug" was removed with a tuberculin syringe and an 18 gauge needle, aspirated into 5 ml of Hanks' balanced salt solution (BSS) in a test tube and capped. The specimens were centrifuged at 1,500 RPM in a table-top centrifuge for 5 minutes, decanted, and 2 ml of hypotonic 0.5% KCl solution was added with gentle agitation to resuspended the cells. The specimens were then placed in a 37°C water bath for 20 minutes in order to swell the cells. Following centrifugation for 5 minutes at 1,500 RPM, the supernatant was decanted and 2 ml of fixative (3:1 absolute methanol:glacial acetic acid) was added. The cells were resuspended in the fixative with gentle agitation, capped, and placed at 4°C for 30 minutes. The specimens were again centrifuged, decanted, 2 ml of prepared fixative was added, and the cells were resuspended and placed at 4°C overnight.

The following day the specimens were again centrifuged, decanted and 0.3 - 0.6 ml of freshly prepared fixative was added to obtain a suitable density. The cells were resuspended and 2 - 3 drops of the suspension were allowed to drop onto a clean, dry slide held at 15° from the horizontal. As the suspension flowed to the edge of the slide, it was ignited by an alcohol burner and allowed to flame. Following ignition, the slides were allowed to dry at room temperature overnight. Duplicate slides were prepared. The slides were stained using a 5% Giemsa solution (Giemsa buffer pH 7.2) for 20 minutes, rinsed in acetone, 1:1 acetone:xylene, and placed in fresh xylene for 30 minutes. The slides were then mounted using Permout (Fisher Scientific) and 24 x 50 mm coverglasses. The coverglasses were selected to be 0.17 mm ± 0.005 mm in thickness by use of a coverglass micrometer. The preparations

were examined using Leitz Ortholux I & II microscopes with brightfield optics and xenon light sources. These specimens were scanned with 10X and 24X objectives and suitable metaphase spreads that were countable were then examined critically using 40X, 63X or 100X oil immersion flatfield apochromatic objectives. Oculars were either 12X or 16X widefield periplanatics and the tube magnification either 1X or 1.25X. The filters used were either a didymium (BG20) or a Schott IL570 m $\mu$  interference filter.

The chromosomes of each cell were counted and only diploid cells were analyzed. They were scored for chromatid gaps and breaks; chromosome gaps and breaks, reunions, cells with greater than ten aberrations, polyploidy, pulverization, and any other chromosomal aberrations which were observed. They were recorded on the currently used forms and expressed as percentages on the summary sheets. Fifty metaphase spreads were scored per animal. Mitotic indices were obtained by counting at least 500 cells and the ratio of the number of cells in mitosis/the number of cells observed was expressed as the mitotic index.

Positive controls in the acute study consisted of animals which had been given the known mutagen Triethylene Melamine (TEM) administered intraperitoneally at a level of 0.30 mg/kg. Negative controls on the acute and subacute studies consisted of the vehicle in which the compound was administered. The dosage levels, solvents and toxicity data are included in the Results and Discussion Section of the report.

b. In vitro study

Human embryonic lung cultures (WI-38) which were negative for adventitious agents (viruses, mycoplasma) which may interfere

were used. These cells were employed at passage level 19. The cells had been transferred using 0.025% trypsin and planted in 32 oz. prescription bottles containing 40 ml of tissue culture medium. When growth was approximately 95% confluent the cells were removed from the glass using trypsin, centrifuged, and frozen in tissue culture medium containing dimethyl sulfoxide (DMSO). Cells were frozen in vials in the vapor phase of liquid nitrogen at a concentration of  $2 \times 10^6$  cells/ml. When needed, the vials were removed from liquid nitrogen, quick-thawed in a 37°C water bath, washed free of DMSO, suspended in tissue culture medium (minimal essential medium [MEM] plus 1% glutamine, 200 units/ml of penicillin and 200 µg/ml of streptomycin and 15% fetal calf serum) and planted in milk dilution bottles at a concentration of  $5 \times 10^5$  cells/ml. The test compound was added at three dose levels using three bottles for each level, 24 hours after planting. The dose levels required a preliminary determination of a tissue culture toxicity. This was accomplished by adding logarithmic doses of the compound in saline to a series of tubes containing  $5 \times 10^5$  cells/ml which were almost confluent. The cells were examined at 24, 48, and 72 hours. Any cytopathic effect (CPE) or inhibition of mitoses was scored as toxicity. Five more closely spaced dose levels were employed within the two logarithmic dosages; the higher of which showed toxicity and the lower no effect. The solvents used and the range finding data are presented in the toxicity data report under Results and Discussion. The dose level below the lowest toxic level was employed as the high level. Logarithmic dose levels were employed for the medium and low levels.

Cells were incubated at 37°C and examined twice daily to determine when an adequate number of mitoses were present. Cells were harvested by shaking when sufficient mitoses were observed, usually 24 - 48



hours after planting, centrifuged, and fixed in absolute methanol:glacial acetic acid (3:1) for 30 minutes.

The specimens were centrifuged, decanted, and suspended in acetic acid-orcein stain (2.0%) and a drop of suspension placed on a clean dry slide. Selected coverglasses 0.17 mm in thickness were placed on the suspension and the excess stain gently expressed from the slide. The coverglasses were sealed with clear nail polish and examined immediately.

The microscopes, objectives, oculars, filters and light sources were enumerated under the metaphase description. Positive controls used were TEM (at a concentration of 0.1 mcg/ml dissolved in saline) and negative controls which consisted of the vehicle in which the test compound was dissolved, which was 0.85% saline. Data were reported on forms currently used and expressed as percentages on the anaphase summary sheets.

### 3. Dominant Lethal Assay

In this test, male and female random bred rats from a closed colony were employed. These animals were 10-12 weeks old at the time of use. Ten male rats were assigned to each of 5 groups; 3 dose levels selected as described above, a positive control (triethylene melamine) (TEM) and a negative control (solvent only). The positive control was administered intraperitoneally. Administration of the test compound was orally by intubation in both the acute study (1 dose) and in the subacute study (1 dose per day for 5 days). Following treatment, the males were sequentially mated to 2 females per week for 8 weeks (7 weeks in the subacute study). Two virgin female rats were housed with a male for 5 days (Monday through Friday). These two females were removed and housed in a cage until killed. The male was rested on Saturday and Sunday and two new females introduced to the cage on



Monday. It has been our experience that conception has taken place in more than 90% of the females by Friday and that the two day rest is beneficial to the male as regards subsequent weekly matings. Females were killed using CO<sub>2</sub> at 14 days after separating from the male, and at necropsy the uterus was examined for deciduomata (early deaths), late fetal deaths and total implantations.

Sufficient animals were provided in our experimental design to accommodate for any reduction in the number of conceptions. Each male was mated with two females per week, and this provided for an adequate number of implantations per group per week (200 minimum) for negative controls, even if there was a fourfold reduction in fertility of implantations. Results were analyzed according to the statistical procedures described in Supplementary Materials and Methods. Corpora lutea, early fetal deaths, late fetal deaths and total implantations per uterine horn were recorded on the raw data sheets, which are submitted separately.

D. Supplementary Materials and Methods

1. Host-Mediated Assay In Vitro and Formulae

a. Bacterial in vitro plate tests

This method has been published by Ames: The Detection of Chemical Mutagens with Enteric Bacteria, in Chemical Mutagens; Principles and Methods for Their Detection, Vol. 1, Chapter 9, pp. 267-282, A. Hollaender, Editor, Plenum Press, New York (1971).

b. In vitro for mitotic recombination

(1) Strain D-3 was grown to stationary phase on complete medium agar plates at 30°C (3-4 days). Cells were rinsed from the plates and washed twice in saline and cell concentration determined spectro-

photometrically. (A standard curve previously determined for colony forming units versus % transmittance at 545 mu was easily used.)

(2) Cells from the concentration suspension were diluted appropriately into 0.067 M Phosphate buffer pH 7.2 to provide  $5 \times 10^7$  cells/ml in a total of 25 ml.

(3) The test chemical was first tested for 4 hours at 30°C, with shaking, at concentrations which permitted determination of the 50% survival level. Then, if not included in the first experiment, the compound was tested again only at the 50% survival level. If 50% survival level could not be determined, the arbitrary test level of 5% w/v was used.

(4) Following treatment, cells were diluted and plated on complete agar medium for determination of total population and red sectors. Total surviving population was conveniently measured on plates of  $10^{-4}$  and  $10^{-5}$  dilutions using 0.2 ml per plate (5 plates), and sectors determined on plates of  $10^{-3}$  and  $10^{-4}$  dilutions using 0.2 ml per plate (5 plates). Plates were incubated for 2 days at 30°C followed by a holding period of 2 days at 4°C to promote color development with limited enlargement of the colonies. Red sectors were scored by systematically scanning the plates with a dissecting microscope at 10X magnification.

(5) The frequency of red sectors can then be calculated and may be expressed conveniently as sectors per  $10^5$  survivors for comparison with untreated controls.

(6) Ethyl Methane Sulfonate (EMS) was employed as the positive control in both in vitro systems.

c. Minimal medium (bacteria):

Spizizen's Minimal Medium:



4X Salt Solution:

$(\text{NH}_4)_2\text{SO}_4$	8.0 gm
$\text{K}_2\text{HPO}_4$	56.0 gm
$\text{KH}_2\text{PO}_4$	24.0 gm
Na Citrate	4.0 gm
Mg $\text{SO}_4$	0.8 gm
Biotin	0.004 gm
$\text{H}_2\text{O}$	qs to 1 liter Sterilize by autoclaving (121°C/15 min.)

Medium:

4X Salt Solution	:250 ml	
5.0% Glucose (sterile)	:100 ml	(If histidine is added at concentration of 30 mg/liter, this becomes a complete bacterial medium.)
1.5% Bacto-agar (sterile)	:650 ml	

d. Complete medium (bacteria):

Bacto-Tryptone	1.0 gm
Yeast-Extract	0.5 gm
Bacto-Agar	2.0 gm
Distilled $\text{H}_2\text{O}$	100.0 ml

Sterilize by autoclaving (121°C for 15 minutes).

e. Complete medium (yeast):

$\text{KH}_2\text{PO}_4$	1.5 gm
$\text{MgSO}_4$	0.5 gm
$(\text{NH}_4)_2\text{SO}_4$	4.5 gm



Peptone	3.5 gm
Yeast-Extract	5.0 gm
Glucose	20.0 gm
Agar	20.0 gm
Distilled H <sub>2</sub> O	1000.0 ml

Sterilize by autoclaving (121°C for 15 minutes).

2. Cytogenetics In Vitro Preparation of Anaphase Chromosomes  
(from Nichols, 1970)

"Anaphase preparations may be made by several methods. One convenient approach is to grow cells directly on coverslips in petri dishes. With human fibroblasts 400,000 cells added to a 22 x 44 mm coverslip in a 50 mm petri dish grown in a 5% CO<sub>2</sub> atmosphere in air has proved very satisfactory. When adequate numbers of mitoses are visualized directly utilizing an inverted microscope (usually 48 to 92 hours after planting) the coverslip is transferred to absolute ethanol for 15 minutes for fixation. They are then stained with any one of a number of suitable stains (Fueigen, May-Grunwald-Giemse, orcein) and attached to a slide with mounting media for evaluation. Anaphase preparations may also be prepared on cells grown in suspension or cells from a monolayer that have been put into suspension. In this instance the cells are centrifuged and fixed with the squash fixative. They are then suspended in the stain and a drop of the suspension put on the slide and covered with a coverslip. However, in this case, only the excess stain is gently expressed from under the coverslip and no squashing is carried out. In anaphase preparations no pretreatment with colchicine or hypotonic expansion is used and no technique for spreading the cells is used, so that the spindle and normal relationships of the chromosomes are not disturbed."



### 3. Statistical Analyses of Dominant Lethal Studies

The following statistical analyses were employed as a means of analyzing the results of the dominant lethal studies.

#### a. The fertility index

The number of pregnant females/number of mated females with the chi-square was used to compare each treatment to the control. Armitage's trend was used for linear proportions to test whether the fertility index was linearly related to arithmetic or log dose.

#### b. Total number of implantations

The t-test was used to determine significant differences between average number of implantations per pregnant female for each treatment compared to the control. Regression techniques were used to determine whether the average number of implantations per female was related to the arithmetic or log dose.

#### c. Total number of corpora lutea

The t-test was used to determine significant differences between average number of corpora lutea per pregnant female for each treatment compared to the control.

#### d. Preimplantation losses

Preimplantation losses were computed for each female by subtracting the number of implantations from the number of corpora lutea. Freeman-Tukey transformation was used on the preimplantation losses for each female and then the t-test was used to compare each treatment to control. Regression technique was used to determine whether the average number of preimplantation losses per female was related to the arithmetic or log dose.

e. Dead implants

Dead implants were treated the same as pre-implantation losses.

f. One or more dead implants

The proportion of females with one or more dead implants was computed, each treatment compared to control by chi-square test and Armitage's trend used for linear proportions to see if proportions were linearly related to either arithmetic or log dose. Also, probit regression analysis was used to determine whether the probit of the proportions was related to log dose.

g. Two or more dead implants

The proportion of females with two or more dead implants computed was treated same as above (f).

h. Dead implants per total implants

Dead implants per total implants were computed for each female and used Freeman-Tukey arc-sine transformation on data for each female; then used t-test to compare each treatment to control.

Historical control data was compiled on a continuous basis as studies were completed. In addition to comparing each treatment to control, as outlined above, each treatment was compared to a historical control.

In order to take variation between males into account, a nested model was used. An analysis of across weeks is also provided.

In addition to these tests, the distribution forms of the various parameters were tested in order to evaluate the appropriateness of some of the tests being used. Certain correlations between parameters may exist and were examined as one step to determine the appropriateness of models. If necessary, alternate test methods were implemented.

The results are presented in tabular form with the addition of historical control information. In addition to these tables, a written report of all findings is provided. As information became available from the on-going investigation of these data, it was reported and suggestions included for changes to the methods of analysis. The statistical reports give the level of significance using both a one-tailed and two-tailed test. Finally, a summary sheet for each study is provided.



M O D E L

$$y_{ijk} = \mu + \alpha_i + \epsilon_{ij} + e_{ijk}$$

$i = 1, 2$  Group

$j = 1, 2, \dots, 10$  Males within each group

$k = 1, 2$  Females within Males within Groups

ASSUMPTIONS:

$$\alpha_1 + \alpha_2 = 0, \quad \epsilon_{ij} \sim \text{nid}(0, \sigma_\epsilon^2)$$

$$e_{ijk} \sim \text{nid}(0, \sigma^2)$$

Males are randomly drawn from infinite population

S. U.	d. f.	S. S.	MS	E(MS)	F
TOTAL	39	$\sum\sum\sum (y_{ijk} - \bar{y}_{...})^2$			
GROUPS	1	$20 \sum (\bar{y}_{i..} - \bar{y}_{...})^2$	$S_1^2$	$\sigma^2 + 20\sigma_\epsilon^2 + 20\sigma^2$	
MALES WITHIN GROUPS	18	$2 \sum\sum (\bar{y}_{ij.} - \bar{y}_{i..})^2$	$S_2^2$	$\sigma^2 + 2\sigma_\epsilon^2$	
REMAINDER	20	$\sum\sum\sum (y_{ijk} - \bar{y}_{ij.})^2$	$S_3^2$	$\sigma^2$	

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F. Abbreviations

1. mu = micron
2. mcg = ug = microgram
3. g = gram
4. kg = kilogram
5. ml = milliliter
6. rpm = revolutions per minute
7. °C = degrees centigrade
8. pH = power of the hydrogen ion concentration to the base 10
9. M = molar solution
10. conc. = concentration
11. MTD = maximum tolerated dosage = High = LD<sub>5</sub> if determined or else exceedingly high dose, such as 5 g/kg
12. INT = intermediate = medium level
13. USE = usage level if known = low level
14. BSS = balanced salt solution
15. C-metaphase = cells arrested in metaphase, using colchine or colcemid
16. LD<sub>50</sub> = that dosage which produced 50% mortality in the group of animals treated
17. LD<sub>5</sub> = that dosage which produced 5% mortality in the group of animals treated
18. NC = negative control
19. PC = positive control
20. AU = acute usage level (low level)
21. AI = acute intermediate level (medium level)
22. AMTD = acute maximum tolerated dose level (LD<sub>5</sub> level, high level)



23. SAU = subacute usage level (low level)
24. SAI = subacute intermediate level (medium level)
25. SA LD<sub>5</sub> = subacute LD<sub>5</sub> level (MTD level, high level)
26. CO<sub>2</sub> = carbon dioxide
27. DMN = Dimethyl nitrosamine
28. EMS = Ethyl methane sulfonate
29. TEM = Triethylene melamine
30. DMSO = Dimethyl sulfoxide
31. MEM = minimal essential medium (Eagle's)
32. CPE = cytopathic effect
33. his = histidine marker
34. D-3 = mitotic recombinant strain of Saccharomyces
35. mf = mean mutant frequency
36. MFt/MFc = mean mutant frequency of the test compound group compared to mean mutant frequency of the negative control group
37. CFU = colony forming units
38. WI-38 = code name for a strain of human embryonic lung tissue culture cells
39. Rec x 10<sup>5</sup> = mitotic recombinants x 10<sup>5</sup>
40. Mean B/A = mean frequency
41. tot. scr. = total scored
42. tot. = total
43.  $\chi^2$  = a test of variation in the data from the computed regression line - tested in these studies at the 5% level
44. Aber. = aberrations
45. Frag. = fragment
46. HMA = host-mediated assay

